REPRODUCTIVE PERFORMANCE, EARLY PROGENY WASTAGE, AND CERVIX RESPONSE USING FIXED-TIME INTRAUTERINE OR TRANSCERVICAL INSEMINATION OR NATURAL SERVICE FOLLOWING SYNCHRONIZATION OF ESTRUS AND OVULATION IN GOATS

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LIST OF ABBREVIATIONS

AI	Artificial insemination
ANOVA	Analysis of variance
AMDUCA	Animal medicinal drug use clarification act
ART	Assisted reproductive technologies
CG	Chorionic gonadotropin
CI	Confidence interval
CIDR	Controlled intravaginal drug releasing
CL	Corpus luteum
CP	Crude protein
CR	Conception rate
DIM	Days in milk
DNA_{mt}	Mitochondrial deoxyribonucleic acid
dPB	Days post-breeding
E/OS	Estrus ovulation synchronization
eCG	Equine chorionic gonadotropin
EPL	Early pregnancy loss
F	Fertility
Fc	Fecundity
FGA	Fluorogestane acetate

FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
hCG	Human chorionic gonadotropin
IM	Intramuscular
IV	Intravenous
kDa	1000 Daltons
KR	Kidding rate
LAI	Laparoscopically-aided intrauterine artificial insemination
LH	Luteinizing hormone
LR	Likelihood ratio
LSM	Least squares means
MAP	Medroxyprogesterone acetate
MGA	Melengestrol acetate
MHz	Megahertz
mL	Milliliter
NSp	Natural service pen
OLS	Ordinary least squares
OR	Odds ratio
P	Probability
P4	Progesterone
$PGF_{2\alpha}$	Prostaglandin
PPL	Post-partum loss
PPNL	Pre/peri-natal loss

PR	Pregnancy rate
Pr	prolificacy
r	Pearson's product-moment correlation coefficient
r^2	Coefficient of determination
RER	Relative error rate
RET	Reproductive efficiency traits
RP	Reproductive performance
RR	Risk ratio
RRR	Risk ratio range
SMB	Syncro-Mate-B (Norgestomet)
TrAI	Transcervical insemination
UI	Ultrasound imaging
W	Wald statistic
χ^2	Chi-square

Foreword

Chapter III, has been submitted to the Journal of Clinical Theriogenology and is in the process of being revised. The original submission was drafted with the following citation: Loetz, E., L. Dawson, J. Hayes, I. Portugal, and J. Malayer. (2012). "Assessment of diagnostic validity of pregnancy diagnosis and fetus number determination by ultrasound imaging in mixed parity dairy and meat/fiber goats." *Clinical Theriogenology.*

The material of Chapter IV has been presented in poster format at the International conference of ruminant reproduction and the abstract has been published and cited in the following format:

Loetz, E., L. Dawson, J. Hayes, I. Portugal, T. Sahlu and J. Malayer. (2011). Reproductive performance in goats following estrus and ovulation synchronization with different progesterone time exposure, gonadotropins (eCG, hCG) and fixed-time intrauterine or transcervical insemination or natural service. Reproduction in Domestic Ruminants VII. J. L. P. MC Lucy, M.F. Smith and T.E. Spencer. Anchorage, Alaska, Nottingham University Press**:** 621.

Chapters V and VI of this dissertation will be submitted to peer-reviewed journals for publication consideration. Both chapters have been formatted and presented in this dissertation in the style required by the Journal of Theriogenology.

CHAPTER I

INTRODUCTION

Economic relevance of the goat industry in the U.S. and regionally

The goat industry is the fastest growing agricultural industry in the nation.[1] The country is the top goat meat importer in the world with 18% of the total world market imports.[2] Regionally the Central Southwestern States harbor the largest goat population (2.2 M) which represents 70% of the total U.S. inventory.[3]

Reproductive performance

Here, we define reproductive performance (RP) as the ability to produce a given number of offspring within a specific span of time. Genetics, the environment and their interactions influence RP. Improvements in RP will only result from better understanding of the physiological events leading to estrus, ovulation, fertilization, establishment of pregnancy and parturition.

Reproductive management

Reproductive efficiency characteristics are quantitative genetic traits governed by many genes with low heritability[4, 5] and are subject to substantial modification by environmental factors including management actions.

The seasonal nature of breeding remains the major constraint to reproductive management of goats in the U.S.[6] Insufficient data to describe RP of domestic meat goat breeds in representative environments hampers the ability to gauge progress.

Improved herd genetics can be developed, in part, using assisted reproductive technologies (ART), which can also reduce labor inputs. Together, these two options can result in improvement of revenue in a commercial setting. The biologic component of this objective can be fulfilled by artificial insemination (AI) using thawed-frozen semen from commercially available high genetic merit sires; to take advantage of economies of scale when using an AI program, hormone based estrus/ovulation synchronization (E/OS) protocols combined with fixed-time AI may be implemented to control female reproductive physiology and reduce labor needs and associated costs.

Assisted reproductive technologies

Despite the potential benefits[7] the adoption of ART is hampered by the caveat that these techniques currently lower the RP of healthy, non-stressed[8] and reproductively sound goats receiving appropriate care.[9-12] Hence, the challenge is to develop strategies to minimize negative effects as compared with natural mating using a fertile buck with females expressing spontaneous estrus.

Research hypotheses

The pivotal hypothesis of this research is that E/OS using short-term progestagen exposure and concurrent chorionic gonadotropins of equine (eCG) and human (hCG) origin interact adversely with fixed-time breeding decreasing RP. Furthermore we hypothesize the source of this antagonistic effect influences the reproductive tract by a

yet unknown mechanism curtailing complete cervical relaxation. Hence, transcervical artificial insemination (TrAI) becomes more difficult with a concomitant increase in progeny wastage, particularly early prenatal losses.

Experimental design

This research was designed as a randomized experimental prospective trial with a two year field-work as well as a clinical trial for laparoscopically-aided breeding procedures. The organization to accomplish this research was distributed into four study components.

Progression of research component studies

Study 1

An early indicator in determination of overall RP is pregnancy. Commonly used methods to diagnose pregnancy are ultrasound imaging (UI) and measurement of blood plasma progesterone (P4) concentration. In small ruminants analysis of plasma P4 concentration has been fully validated for pregnancy determination. On the contrary, despite the fact that UI has been used extensively with goats,[13-19] results have not been consistent or confirmed under non-clinical conditions,[20-23] much less when using non-tractable goat production phenotypes and/or parity categories. Therefore, the initial effort of this research has been to evaluate field UI results of goat pregnancy diagnosis and estimation of fetus number to determine accuracy, precision and robustness.

Study 2

The second research component aimed to quantitatively characterize RP. To our knowledge, no large-scale study evaluating RP of goats using ART in the Southwestern environment of the U.S. has been published. Likewise the short-term (5-6 day) P_4

priming protocol with the simultaneous use of eCG/hCG as a substitute for E/OS protocols based on 12 to 14 d P4 exposure using eCG alone, has not been addressed.

Study 3

There is scarce published information regarding prenatal and perinatal losses in local goats. For this reason, the third component of this research was designed to characterize and evaluate the extent that early progeny wastage can be credited for lowering RP.

Study 4

Furthermore, our previous unpublished TrAI work indicated that short P4 exposure altered the site of insemination. Hence, in the final study component we postulate that short P4 priming, in combination with eCG and hCG, interferes with optimum cervical relaxation hampering complete penetration of the AI instrument and/or prolonging the time it takes to get through the uterine cervix.

Objectives

In summary, this research aims to provide improved validation of ultrasound imaging technology for pregnancy diagnosis in different goat production phenotypes, and to establish the influence of E/OS in different goat breeds. Particularly we seek to determine the influence of time of exposure to P4 and dose of eCG and hCG on reproductive efficiency traits, early progeny wastage, and time required to traverse the uterine cervix in goats bred at a fixed-time by natural service or artificial insemination.

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CHAPTER II

LITERATURE REVIEW

Background – Goat historical and economic perspective

Goat historical perspective

Archeological remains[1] and anthropological studies[2] indicate that 10,000 years ago goats (*Capra hircus*), in the form of their wild progenitor the Bezoar (*Capra aegagrus*), was one of the first ungulates to be domesticated by man.[2, 3] those findings have been partly confirmed by analysis of molecular data generated from DNA_{mt} haplogroups.[4]

The historical background of goat production in the U.S. has been addressed in detail elsewhere.[5-8] In summary, goats arrived in the Americas with the Spanish conquest more than 500 years ago. In time Spanish colonies were established in North America and it is likely that Caribbean livestock was brought through Florida, and into the southeastern part of the continent in what is now Mississippi, Alabama, and Georgia. Farther west, goats probably came with the first Spanish religious missionaries in their treks to Baja and Central California and on to Texas, Arizona, New Mexico and the rest of the Central Southwestern region including Oklahoma.

In an effort to improve productivity, exotic dairy (Alpine, Saanen, Oberhasli, La Mancha, Toggenburg and Nubian), fiber-producing (Angora and Cashmere), and meat breeds

(Boer and Kiko) have been introduced. Equally important in the effort to increase production have been the cross-breeding strategies to produce composite breeds.[9]

Goat economic perspective

In similar fashion as found in dairy cows[10-13] reproductive performance (RP) is the largest determinant of income in a livestock enterprise.[14-17] Economic relevance of reproduction and profitability in a goat enterprise, and ultimately its economic viability depends on the reproductive and maternal abilities of the doe herd.[18] The major constraints to reproductive management of goats in the U.S. remain, as pointed out a decade ago, the seasonal nature of breeding, and a lack of data on the RP of domestic meat goat breeds.[19]

Actual cost of financial losses due to deficient RP has not been determined for goats. Nonetheless, the negative impact of inefficient reproductive programs is presumed to be considerable. Most information available emphasizes costs of production and is available through agricultural extension services.[20-25] However this information is specific to certain regions and cannot be generalized. Economic consequences due to impaired RP are not apparent because reproductive inefficiency deals with unattained production. For this reason attempts to establish the cost of ineffective RP has centered on identifying reproductive management factors pivotal in determining the herd's reproductive productivity. That is, seasonality, age at first breeding, estrus detection efficiency, pregnancy rate, prolificacy, kidding rate, reproductive disorders and unforced culling.

Goat Inventories – United States, Central Southwest region, and Oklahoma

The total number of goats in the U.S. according to the most recent inventory available[26] is 3.1×10^6 , up by 5% from the 2.9×10^6 published for the year 2007.[27] Total goat inventory

reports show Oklahoma as being number 4 in the nation with 91×10^3 meat and 6×10^3 dairy goats[26] after Texas (2×10^6), Tennessee (170.5×10^3) and California (138×10^3) in the goat inventory ranking.[28] The Central Southwest region (Arkansas, Louisiana, Oklahoma, and Texas)[29] is the most important goat producing section in the country with a total of 2.2×10^6 goats which represent 70% of the total inventory.

Goat industry and marketing in the United States

In the current globalized marketing context, the goat industry in the U.S. represents an emerging type of livestock agribusiness characterized as "a non-traditional, alternative agricultural enterprise."[18] Two relevant facts, regarding present day goat production in the U.S. need to be emphasized to convey the actual national goat industry status and marketing outlook. First, the goat industry is the fastest growing agricultural industry in the nation. In fact, estimates of the total goat market in the U.S. establish that the industry is growing at a rate of 10 to 15% annually[6] with an increased meat goat inventory of 527%. [26, 28] Second, despite the robust growth of the caprine industry, the U.S. remains a net importer of goat products. Imports of goat meat have risen from approximately 3×10^6 tons in 1990 to 12.6×10^6 tons in 2001.[30] More recent data indicates that U.S. is now the top goat meat importer in the world with 18% of the total world market imports.[31]

Reproductive characteristics of the female goat

Goats are a polytocous small ruminant species bearing litters from 2 to 6 offspring depending on the breed. Goats like sheep are classified as seasonally polyestrous and are short-day breeders.[32] Depending on various factors (i.e., genotype, environment and their interaction) goats become sexually active usually in the first breeding season of their life.[19]

Age at puberty can vary. For example, the Shiba breed reaches puberty at an average (± SE) of 27.0 ± 0.9 weeks when this breed reaches 12.2 ± 0.5 kg of body weight.[33] Representative contemporary U.S. breeds attain puberty when they reach 27 to 32 kg or 30-50% of adult body weight with a range in age from 12 to 28 weeks of age.[34]

Goats have a potential productive and reproductive life span of approximately 10 to 12 y. which is seldom reached because management decisions to replace animals in a herd are motivated largely by economic rather than biologic considerations.

In the Central Southwestern U.S. goats are reproductively anestrous during February through September. Transitional periods, between anestrous and estrous expression, and vice versa, occur during July and August (pre-cycling) and January (pre-anestrum). In the breeding season, goats exhibit on the average a 21-day estrous cycle, expressing sexual receptivity (estrus) for approximately 18 to 24 hours. Each estrous cycle has a follicular phase of 16 to 18 days when metestrus and diestrus occurs and a luteal phase of 3 to 5 days when proestrus and estrus ensues. Goats ovulate spontaneously with an average of three or four follicular waves during the follicular phase of the estrous cycle. Ovulation occurs about 12 h after the end of estrus with fertilization taking place in the upper one third of the oviduct. If ova are fertilized one or more embryos will enter the uterus around day 5 and 6 similar to what has been observed in the cow.[35, 36]

Maternal recognition of pregnancy in the goat occurs around day 15 and 17[37] while embryo attachment to the uterine endometrium begins on day 18.[38] Recognition of pregnancy is mediated by an embryo hormonal signal believed to be two trophoblastic proteins (caIFN$_t$) with MW of 17 and 22 to 24 kDa, respectively.[39]

Gestation in the goat lasts approximately 150 d, and has been divided into pre-attachment (from fertilization to day 18) and post-attachment (from day 18 to day 40) embryonic phases and fetal development (from day 41 to term).

Goat reproductive physiology and endocrinology has been reviewed previously[19, 32, 40, 41] and is generally similar to that observed in sheep and cows, however details vary in several aspects. Post-puberty reproductive events are governed by an interaction with photoperiod through the optic tract and epithalamus.[42]

An increase in dark hours triggers the release of pineal melatonin. Melatonin secretion is required to stimulate hypothalamic nuclei gonadotropin-releasing hormone (GnRH) secretion. In short day breeders such as the goat, melatonin is synthesized and secreted during the night hours when it is converted from serotonin through neural path circuits.

Secretion of GnRH is pulsatile and occurs in two modes, a tonic mode that controls gonadotroph cells in the pituitary, and a surge mode that triggers an LH spike necessary for ovulation. LH also promotes luteinization of ovarian theca cells for the production of P4 by the corpus luteum.[43, 44]

Once the breeding season is initiated there is hypothalamic-pituitary-gonadal axis control of estrous cycles through reproductive hormones (e.g., hypothalamic liberating factors, gonadotropins and gonadal steroids) that interact in concert through various feedback mechanisms. Hypothalamic GnRH also stimulates the anterior pituitary (adenohypo-physis) to release follicle stimulation hormone (FSH) in a wave pattern that occurs 3 to 4 times per estrous cycle. The release of FSH stimulates the production of estrogen and inhibin as well as promotes follicular growth. When estrogen reaches a certain

concentration goats express ethological estrus with associated characteristic behavior. Inhibin acts as a negative feedback to inhibit the release of FSH from the anterior pituitary. A high concentration of P4 has a negative feedback effect on the brain and hypophysis down-regulating genes that transcribe GnRH, FSH and LH.

Progesterone also suppresses estrus behavior and prepares the uterus for pregnancy if fertilization of the ovum by the spermatozoon has taken place. Failure to establish pregnancy around day 15 to 16 after fertilization has taken place triggers the secretion of uterine prostaglandin-$F_{2\alpha}$ which is responsible for the regression of the CL and the resumption of estrous cycles.[45-47] If maternal recognition of pregnancy takes place the embryo attaches to the endometrium, continues developing and pregnancy is established with P4 secreted continuously.[48] At this point the presence of P4 will increase gland development in the uterus as well as in the mammary gland and prevent uterine PG production until parturition.[49]

Goat reproductive performance in the Central Southwestern region of the U.S.

Recently researchers have reviewed factors affecting goat meat and milk production and quality.[9, 50] However, review of the factors affecting reproduction[51] which directly influence all production traits is limited. What has been published comes from herds receiving experimental treatments. The scarcity of data available regarding basal levels of reproductive performance in representative eco-systems curtails necessary comparisons.

Although there is a vast collection of reproductive research studies, to date few studies have addressed goat performance in the U.S. using large herds and a wide variety of locally representative breeds and/or production phenotypes evaluated under similar

environmental influences. One notable exception is work performed by Richard Browning's group at Tuskegee University,[18, 52-54] with the important caveat that herds studied over the years have all been herds bred by natural service. Fewer yet is the information available pertaining goat production specifically for the Central Southwest region such as that generated over 17 years ago.[55]

During the past 50 years, there has been a negative direct relationship between the fertility of high-producing lactating dairy cows and increases in milk production, where data shows that conception rates to first service of lactating cows reached 32% and in heifers it remained above 50%.[56] Similarly, over the past two decades, the United Kingdom has seen calving-to-first-service rates decrease from approximately 60% to 40%.[57, 58] In the U.S. lactating cows have experienced a decline to 32% in calving-to-1st-service rates.[59]

Reproductive trends cannot be prognosticated from the published data available. Though, from existing information concerning other farm ruminant species, it is possible to anticipate that over the years fertility of milking goats may have dropped following the same pattern as found for dairy cows. In dairy cows, selection to achieve maximum milk production with little interest on improving reproductive traits have resulted in decreasing trends in fertility rates. A similar scenario may be anticipated to have occurred in dairy goats as well.

A similar pattern is evidenced when meat production is considered. Evaluation of three meat goat breeds in Central Southeastern U.S. revealed that some measures of RP of the

Spanish and Kiko breeds were better than Boer goats.[52] The implication here is also that a breed more intensely selected for meat production alone will have RP compromised.

Consequently, until species specific data is generated and/or selection schemes include reproductive efficiency traits, we can infer that more intensive management for production (e.g., milk, meat) could negatively impact reproductive performance as it has been documented for other species.

Goat reproductive management

Management of goat production phenotypes for meat, dairy and the fiber goat industries have been studied and reported by other authors.[30, 31, 60-62] The practical implication of these issues is that goat production phenotypes are managed differently [9, 50] and this influences the manner in which reproductive management is typically conducted for each group.

Meat and/or fiber producing goat breeds are usually managed under an extensive type of management with access to improved pastures, sheltering and mineral/vitamin supplementation. Dairy herds receive closer attention in a semi-extensive type of management with partial confinement. Often milking animals are housed in a barn and depending on the herd size many producers have adopted machine milking. Feeding is based on high quality pastures and nutritional requirements for milk production are satisfied by mineral/vitamin supplements and concentrate feeds high in calories and protein.

Present operating conditions in commercial U.S. goat production aim to attain several objectives simultaneously. That is, there is interest in maintaining high selective pressure

on genetic traits that improve productivity and market value by implementing reproductive management strategies for maximum reproductive efficiency, while decreasing costs to get females bred. Using the current technology available and concurrently meeting all the objectives emphasized will require use of transcervical artificial insemination by fixed-time breeding as a management tool.

To attain fixed-time breeding, caprine estrus synchronization and ovulation induction is done increasingly by administering synthetic hormones.[63] Although the variety of hormones available for reproductive management has remained relatively unchanged over the last 25 years, legal access and use of some products has changed. Implementation of E/OS protocols have been quite dynamic and, among various other factors respond to breeding either cycling (in season) or anestrous (out of season) goats.

The combination of products (progestagens and gonadotropins), the dose administered, the time of exposure to the pharmaceutical (progestagens), and the order in which each product is used has kept changing over the same time span.[64-70] The result of these non-concerted efforts is that, to date, there is no unanimity on what is the most appropriate E/OS procedure to use, much less consensus on results expected.

There is ample information that has been generated and published on the use of eCG as a reproductive management tool as part of the protocol to synchronize goat estrus and ovulation particularly with out-of-season programs. i.e., breeding in February through August in Northern latitudes. The favorable results obtained with eCG as a component of E/OS protocols has made its use very popular outside the U.S. where its use is not banned. In contrast, the use of hCG in the U.S. is illegal.

Principal mode of action of the major estrus/ovulation synchronization hormones

The mode of action of the major synchronization (E/OS) hormones is straight forward. When goats are reproductively active, exogenous provision of a luteolytic agent will prematurely cause the regression of existing corpora lutea,[71-73] whereas progestagens are supplied to extend the luteal phase.[74, 75]

The extension of the luteal phase by artificially providing exogenous P4 or its analogs is accomplished by their negative feedback influence on the hypothalamus and hypophysis which down regulates the expression of hypophyseal gonadotropins (i.e., FSH and LH).[76, 77] Chorionic gonadotropins, which are not produced by ruminant animals, are supplied to take advantage of their FSH and LH-like biologic effect[78] and because of their luteotrophic effect which has been demonstrated for diverse other species[79-84] including goats.[85]

Breeding procedures

Goats in the U.S. are bred primarily by natural service. When artificial insemination is used, particularly in dairy herds, the most common technique used is TrAI. Laparoscopically-aided intrauterine insemination (LAI), although capable of generating pregnancy rates close to those obtained by natural service,[86] is seldom the procedure of choice because of its high cost, the need for more sophisticated level of expertise, and the fact that LAI is regarded a minor surgical procedure requiring animal pharmacological sedation using controlled drugs which must be prescribed and used under the responsibility of a licensed veterinarian.

Fixed-time breeding

Fixed-time breeding refers to the reproductive management procedure that uses natural or hormonal means to synchronize ovulation and estrus but breeding is programmed to occur at within a predetermined time frame entirely omitting estrus detection. The appropriate span of time for breeding, that increases the probability of maximum fertilization, appears to be protocol-dependent and is presently an on-going research effort.

In terms of reproductive management economic efficiency, fixed-time breeding is the logical practical extension of efforts to synchronize estrus and ovulation for the main reason that eliminates labor input necessary for implementing an estrus detection program and increases the number of females bred in a given amount of time.

Procedures for hormonal estrus/ovulation synchronization

Estrus/ovulation synchronization hormones

A compilation of E/OS reproductive hormones used over the years in goat breeding management is presented in Table 1. These can be classified by various means, including source, target organ, mode of action, chemical and biochemical classification.

U.S. regulation on the use of hormones. Current U.S. regulation allows veterinarians to employ some pharmaceutically prescribed hormones under the "extra-label" use strategy as specified under the AMDUCA - FDA Compliance Policy Guide.[87]

Table 1. Classification of reproductive hormones used in estrus/ovulation synchronizing protocols.

Name (abbreviation)	Origin	Target Tissue	Chemistry	Biochemical Classification	Commercial Source	Primary Action
Gonadotropin releasing hormone (GnRH)	Arcuate nuclei of the thalamus; hypothalamic surge and tonic centers	Gonadotroph cells of the Adenohypophysis	Decapeptide; I	Neuropeptide	Cystorelin	Release of hypothalamic gonadotropins → FSH + LH
Progesterone (P4)	Uterus, ovary, embryonic membranes	Hypothalamus, uterine endometrium and myometrium, mammary gland	Steroid	Progestagen	CIDR, Norgestomet	Support pregnancy, endometrial secretion, GnRH release inhibition, sexual behavior inhibition
Estradiol (E_2)	Ovarian theca and granulosa cells	Hypothalamus, entire reproductive tract, mammary gland	Steroids	Estrogens	Estrumate (estradiol benzoate)	Onset of sexual receptivity, promotes GnRH release, elevated secretory activity of entire reproductive tract, enhanced uterine motility
Equine chorionic gonadotropin (eCG), Human chorionic gonado-tropin (hCG)	Chorionic girdle cell in the mare's placenta or syncytiotrophoblast cells of human chorionic placenta	Ovary	Glycoprotein	Gonadotropin	Folligon (eCG) Chorulon (hCG) PG600 (eCG+hCG)	Maintenance of corpus luteum at the beginning of pregnancy, facilitates ovarian P4 production, formation of accessory corpora lutea
Prostaglandins ($PGF_{2\alpha}$)	Vesicular glands of uterine endometrium	Corpus luteum, uterine myometrium, ovulatory follicles	Eicosanoid (C-20 fatty acid)	Prostaglandins	Lutalyse, Pluset	Luteolysis, promotes uterine tone and contraction, ovulation
Other associated reproductive hormones						
Luteinizing hormone (LH)	Gonadotrope cell of the adenohypophysis	Ovarian theca interna and luteal cells	Glycoprotein	Gonadotropin	Lutropin-V	Ovulation, luteinization of ovarian cells to luteotropes →P4
Follicle stimulating hormone (FSH)	Gonadotrope cell of the adenohypophysis	Ovarian granulose cells	Glycoprotein	Gonadotropin	Folltropin	Follicle maturation → E_2
Melatonin (none)	Pinealocytes of Pineal gland, retina, GI tract	Hypothalamus, pars tuberalis of pituitary	N-acetyl-5-methoxytrypt amine	Amine-tryptophan indoleamine	Regulin	Responds to changes in photoperiod which triggers cyclic sexual receptivity, regulates circadian rhythms

Estrus/ovulation synchronization procedures. Over the years various authors have reviewed a number of E/OS protocols used in goat reproduction for synchronization of cycling goats:[42, 69, 74, 88-90] and for estrous induction and synchronization outside of the breeding season.[91] A recent review on hormonal means of E/OS[63] has addressed the more prevalent current pharmacologic protocols used. Nonetheless, it is the purpose of this literature review to highlight procedures that have used eCG and hCG concurrently (i.e., PG600) as this is the only practical strategy in keeping with U.S. laws and regulations.

Behavioral and/or physiologic response promoted by E/OS protocol. The different E/OS protocols reported[74] have many similarities, although they can elicit very different responses. Significant variability in result is found even among animals of the same breed, age, weight, parity, number of breedings and type of breeding procedure.

The common features of E/OS protocols used in goats are as follows. First, with the intent that all animals start at the same stage of the estrous cycle, a luteolytic dose is given at the onset. Goats that have a functional corpus luteum (CL) will respond by regressing the CL and a new follicular growth wave will commence; goats with no functional CL will be at one of their follicular waves. Second, a progestagen is given concurrently with or shortly after the aforementioned luteolytic agent. The goat is exposed to the progestagen for a variable amount of time with the intention to allow follicular wave dynamics to proceed but averting behavioral or physiologic estrus for as long as the progestagen is present. Third, if the goats are not cycling or are anestrous an optional dose of chorionic gonadotropin (CG) is administered twenty-four hours before terminating P4 exposure (some E/OS protocols administer CG on the same day that P4 is

removed). The presence of CG will elicit both an FSH and LH-like biologic response. Finally, at the end of the procedure (when P4 is removed) a second luteolytic dose is given to ensure ovulation.

The actual time that goats will exhibit estrus as a result of the E/OS treatment procedure and the number of goats responding both behaviorally and physiologically varies depending on the type of gonadotropin used (data not shown). In general, estrus behavior starts 12 to 48 h after P4 removal depending on protocol used. Ovulation has been shown to occur 60 to 72 h after P4 withdrawal. Fixed-time breeding usually takes place 48 to 50 h after removing the progestagen.

Other E/OS protocols rely on the use of GnRH to promote pituitary gonadotropin secretion instead of the providing CG. This GnRH method, known as the Ovysynch protocol, was developed for cattle synchronization and has been successfully implemented in goats.[88] It consists of an injection of GnRH analog (0.004 mg of Buserelin) followed 7 d later by an injection of a luteolytic dose of $PGF_{2\alpha}$. A second injection of GnRH is given 2 d after the $PGF_{2\alpha}$. Does are inseminated 16 h after the second injection of GnRH.[65]

Luteolytic agent

The most common used luteolytic agent in E/OS protocols is Dinoprost Tromethamine a $prostaglandin_{F2\alpha}$ analog ($PGF_{2\alpha}$) commercialized under various trade names. Prostaglandin is a paracrine or autocrine hormone produced by many organs and tissues in the body.

Pharmacologically prostaglandin has several effects on the female reproductive system, including regression of the CL,[92] increased activity of the myometrium, relaxation of the cervix, and the inhibition of steroidogenesis by corpora lutea.[93] In goats prostaglandins were initially used in reproductive management to cause non-sepsis abortion[94] in an effort to have induced parturitions.

In the normal estrous cycle, regression of the CL is mediated by uterine prostaglandins produced by endometrial cells synthesized from essential fatty acids.[95, 96] Prostaglandin is released from the cell by passive diffusion but also leaves and re-enters the cell by means of a broadly-expressed, 12-membrane-spanning domain integral membrane protein prostaglandin transporter.[97] Target cells contain a variety of prostaglandin G-protein trans-membrane receptors.[98]

It was shown that a dose of 0.0385 mg of $PGF_{2\alpha}$/kg (1.75 mg of $PGF_{2\alpha}$/45.36 kg) was effective for induction of estrus in 100% of the does and P4 levels were reduced below 1 ng/mL within 24 h.[99] In a different study[100] 60 µg $PGF_{2\alpha}$ in 0.1 mL saline, at 9-11 d following estrus or between 28 to 32 d of gestation was luteolytic in both non-pregnant and pregnant goats when injected directly into the CL. Taken together these studies lead to the conclusion that the luteolytic dose required to cause CL regression is much less than the present 2 mg/doe routinely used in E/OS protocols.

Both PG and the chorionic gonadotropins eCG and hCG are given at the end of the E/OS protocol, for this reason the biological and practical significance of their potential interaction and effect on reproductive events is relevant and needs to be considered to explain possible effects of RP. Pharmacokinetics studies have shown that PG is

distributed very rapidly to tissues after injection and is rapidly degraded. Because naturally occurring PGs are rapidly metabolized a number of PG analogs have been developed which are resistant to rapid inactivation.[101, 102] Measurement of their metabolites does not imply that the naturally occurring PG will have the same rate of clearance.

The biological half-life of the principal metabolite of PGF2a in goats, cow and pigs, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in peripheral plasma was 18.6 ±0.74 S.D. m,[103] 7-8 m,[104] and 14.97 ±1.33 S.D. m,[105] respectively. Following intramuscular injection of carboprost (trade name for the tromethamine salts which are synthetic analogs of prostaglandin 15-methyl-$PGF_{2\alpha}$) plasma levels in humans peaked after 20 m and declined slowly thereafter. In amniotic fluid the half-life was between 31 and 37 h.[101]

Progestagens

Progesterone or its functional analogues which are traditionally administered in E/OS protocols for a period similar to the duration of a corpus luteum spurium (14-16 d) are used to thwart estrogen release and, indirectly, LH surges in the follicular and late follicular stages of the estrous cycle,[106, 107] respectively. Hence, the use of progestagens effectively prevents behavioral estrus and postpones both breeding and ovulation until its influence is removed.

The use of P4 and its analogues for blocking or inducing the occurrence of estrus and ovulation have been reviewed for the breeding[108] and non-breeding season,[91] correspondingly. In summary although results of out-of-season E/OS are frequently less than results obtained during the breeding season, the strategies for synchronization

deployed during each season are similar with the difference that chorionic gonadotropins during anestrus is indispensable for a successful response, whereas not essential during the breeding season.

To design and evaluate hormonally synchronized estrus procedures some researchers have focused on ovarian follicle dynamics, rather than corpora lutea lifespan.[67, 109] This action allows for a reduced period of P4 exposure, from 5 to 6 d rather than 14 to 16 d.

Year-round breeding field trials have validated the use of P4 for 5 to 6 d in combination with a luteolytic dose of PG at time of initiation of the P4 regime and eCG or estradiol benzoate (EB)[110, 111, 112] or P4 for 5 d with no CG[113] rather than the traditional 12 to 14 d recommended since its inception[114] or 9 to 16 d as reviewed by Whitley and Jackson (2004)[74] or even the longer 16 to18 d[115] or 18 to 21 d.[65]

The results of using different protocols is not only inconsistently different dependent on the E/OS protocol used but depend on covariates included, if any, (e.g., breed, body condition, age, previous hormonal treatments, nutritional supplementation, time in the reproductive season, type of breeding procedure, breeding technique, etc.) that have not been standardized across studies. In general pregnancy rates from 25% to 90% has been documented.

Recently, Menchaca and co-workers[116] have shown that a 5 to 6-day short-term P4 exposure protocol induces similar P4 concentrations among treated goats and, when used in combination with eCG or EB, results in similar increase in estradiol-17ß as the levels of estradiol-17ß obtained when using the 12-14 d P4 exposure. In addition, the short P4

exposure protocol elicits a comparable luteinizing hormone surge, inducing ovulation in 86.7% of treated females at approximately 60h after the end of P4 exposure.

The use of P4 for prolonged time has been associated with reduced fertility in ewes,[117] increased embryonic mortality, gamete transport hindrance in the female reproductive tract, insufficient follicular maturation, and delayed ovulation.[77, 112, 118]

E/OS protocols with 16 d progestagen exposure time using three different sources of progestogen, medroxyprogesterone acetate (map), Fluorogestone acetate (FGA) sponges and controlled internal drug release (CIDR) have been evaluated.[108] no significant difference was observed with respect to pregnancy rate 40 days after ai (52, 60 and 47% for CIDR, map and FGA groups, respectively)

Gonadotropins

Gonadotropins are a group of glycosylated protein hormones under the control of the hypothalamic gonadotropin releasing hormone (GnRH). Gonadotropins act on cell membrane receptors present largely, but not exclusively on the gonads.[119] they are secreted by gonadotrope cells of the anterior pituitary of vertebrates and by cells in the primate and equine placenta during pregnancy. Although non-gonadal gonadotropin receptors are known to occur across gender, in this document only the female target organs and/or tissues are contemplated (e.g., uterus, oviduct, cervix, blood vessels and mammary gland).[120, 121]

Chorionic gonadotropins (CG) are hormones belonging to the same family as the pituitary gonadotropic follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid stimulating hormone (TSH). Cg are known to occur only in primates and

equids.[122] although goats have not been subject of genomic analysis for cg expression, other farm animals have been clearly shown not to carry the CGß genes in their genomes, including: cows,[123] pigs[124] and sheep.[125]

CG hormones are heterodimers and structurally bicatenary where the chains are not covalently linked.[126] They share a nearly identical α subunit chain, but each has a unique β subunit chain.[78] The β subunit provides specificity for receptor interactions. In fact, it was demonstrated that within a species, the peptide portion of the α subunit was not only essentially identical between the four hormones (*i.e.,* CG, LH, FSH and TSH) but highly conserved from species to species (e.g., primates, bovine, ovine and equine).[127]

The molecular mechanism of action of gonadotropins is initiated when trans-membrane receptors embedded in the surface of a target cell are triggered by binding with the cognate peptide. Gonadotropin receptors are coupled to the G-protein system which transduces signals by the cyclic AMP second messenger system.[78, 128] Additional mechanisms of activation may be present as it has been shown that in primates chorionic gonadotropins are glycosylated to a different extent, and the sugar moieties are important functionally in the initiation of a post-receptor mechanism.[129]

In general the biological role of CG –in the species where they occur– is to first provide an early signal for pregnancy, elicit expression of genes shown to be essential for implantation and influence the development of the receptive endometrium via secreted paracrine signals.[130] After maternal recognition of pregnancy has taken place and the process of implantation has started, CG prevents the disintegration of the transitional ovarian corpus luteum verum and aids in the transformation and maintenance of the

newly formed CL[131] probably triggered by the interaction with the LH/CG receptor. This interaction of gonadotropin and luteinized cells promote sustained P4 secretion during early pregnancy. CG's added exogenously during goat estrus synchronization are believed to exert the same luteotrophic effect.

Additionally, the role of exogenous CG as part of E/OS, has special significance in goats since the caprine pregnancy is completely CL-dependent[132] and goats are known to experience an inordinate amount of extemporaneous early CL regression resulting in embryonic death and the end of pregnancy.[133] For example, depending on the type of EO/S protocol the proportion of does with premature CL regression was 29 and 17%, respectively.[88]

The only available pharmaceutical in the U.S. for use in farm animals which contains chorionic gonadotropin is the commercially offered product PG600®, designed for porcine reproduction.[134] PG600 is a mixture of 67% chorionic gonadotropin of equine origin (eCG) and 33% chorionic gonadotropin of human origin (hCG).

Equine chorionic gonadotropin (eCG). eCG was originally known as pregnant mare serum gonadotropin (PMSG) due to its association with the source from where it was obtained for commercial purification. eCG was used in the U.S. prior to legislation banning its use in farm animals. However, eCG is currently commonly used in other countries where, when applied to domestic farm species other than the horse, at a dose ranging from 400 IU to 1000 IU.[135]

When eCG is used in goats it characteristically elicits both LH and FSH-like activities.[136, 137] Because of this dual biological effect, eCG has been used to induce ovarian follicular

growth, both for enhanced ovulation and for estrus induction and synchronized breeding programs.[138] Several studies have addressed the influence of eCG for synchronizing goat estrus during the reproductive season and inducing ovulation during anestrous.[139-144, 145]

The main drawback associated with the use of eCG is the induction of a progressive refractoriness in the response of females to the biological effect of gonadotropin as a consequence of an immunogenic reaction.[146-148]

Differences between eCG and hCG have been described;[149] whereas separate genes encode the hLH ß and hCG ß subunits, the same equine gene encodes eLH ß and eCG ß. This means that in the horse functional differences between LH and CG are probably driven only by the differences in their oligosaccharide moieties. Such differences, which may be stochastic, could very well be a potential explanation to the highly variable results obtained when using eCG since the degree of glycosylation affects gonadotropin clearance.[150, 151]

Studies of CL lifespan and fertility after hormonal estrus synchronization in goats[133] have indicated that a combination of P4 and eCG was induced estrus but resulted in a high incidence of short luteal lifespan. It is possible that the low kidding rate and high incidence of embryonic loss could be due to the instability of the luteal lifespan generated by the E/OS protocol used.

Human chorionic gonadotropin (hCG). Importantly, PG600 also contains the luteotrophic agent human chorionic gonadotropin (hCG), a peptide hormone produced in pregnancy by the syncytiotrophoblast layer of the primate placenta.[152] Its biological role in humans is to prevent the early disintegration of the ovarian corpus luteum verum. This

protection is accomplished by interacting with the LH/CG receptor to promote sustained P4 secretion. Later in human pregnancy, this gives way to other placental P4 sources. As mentioned previously, in goats, the CL is the sole progestational hormone source throughout pregnancy.[153]

hCG, like eCG, is also a heterodimeric glycoprotein hormone composed of 244 amino acids (36.7 kDa) produced in pregnancy by trophoblastic cells of the developing embryo and later by syncytiotrophoblast layer of the primate placenta.[152] hCG was first reported to be present in blood and urine of pregnant women by Ascheim and Zondek in 1927.[154]

The α subunit of hCG is a 92 amino acid residue polypeptide with two N-linked oligosaccharides encoded by a single gene physically located on chromosome 6q21.1-23.[155] The ß subunit is a somewhat larger polypeptide molecule of 145 amino acid residues encoded by six highly-homologous genes positioned in chromosome 19q.[155, 156]

Following intramuscular administration, an increase in serum CG concentrations may be observed within 2 h. Peak concentrations occur within 6 h and persist for approximately 36 h. Serum CG levels begin to decline at 48 h reaching undetectable levels after 72 h. Chorionic gonadotropin is distributed primarily in the testes and ovaries of the male and female respectively, with small amounts possibly distributing into the proximal tubules of the renal cortex. Blood levels of CG decline in a biphasic manner. The initial phase half-life has been reported between 5.6 and 11 h, whereas the terminal phase half-life has been reported between 23 and 37.2 h. Following i.m. administration of therapeutic doses, approximately 10 to 12% of the dose is excreted in the urine within 24 h.

The clinical role of hCG when used on dairy cows has been recently reviewed.[157] In summary, it has an effect as a potent luteinizing hormone extending the life span of the CL and increasing P4 synthesis. hCG is capable of inducing ovulation and the formation of accessory CJs if applied early in the luteal phase. In cows it has been found that hCG increases the frequency of three-wave dominant follicular cycles. hCG acts on the ovary independently of the pituitary and its effect is longer lasting than that produced by endogenous LH release.[158]

A similar review, in the context of goat reproduction, has not been performed. Nevertheless, the practical application of hCG use in goat reproduction is for its FSH biological effect because in the presence of one or more mature ovarian follicles, ovulation can be triggered at about 50 h after administration. The role of hCG as a potential luteotrophic agent to prevent the disintegration of the ovarian goat corpus luteum verum and promote pregnancy has only been recently considered.

In cows the effect of using hCG when breeding or around breeding time on conception rates has been inconsistent. Some studies show pregnancy rate increases[159] where first service pregnancy rates were higher for the hCG-treated cows compared with saline-treated cows (37.9% vs 23.6%; $P < 0.001$). Other studies either showed no effect with first service conception rates between the control and treatment groups of 46.3% versus 43.6% ($P=0.68$) or 35% for hCG-treated group compared to 35% for the no-hCG control group or, using a different E/OS protocol, a 37% for hCG-treated group compared to 38.0% for no-hCG control group.[160]

In sheep, repeated administration of hCG during the first two weeks after estrus increased serum P4 concentration in pregnant and non-pregnant ewes, but did not influenced lambing rate or number of offspring at parturition (p=0.546).[161] Concerning the use of hCG 5 days after breeding research has shown that pregnancy rate did not differ between non-treated (86.7%) and hCG-treated (70.6%) nulliparous goats or between non-treated (78.3%) and hCG-treated (84.4%) lactating does. Overall no differences in kidding rate was documented for control and hCG-treated goats; 75.0 and 75.7%, respectively.[162]

Combined use of eCG and hCG. In practice the combined use of eCG and hCG could be accomplished by use of the commercially available product PG600, although few published reports have addressed the concomitant use of eCG and hCG in goats. The interest in this gonadotropin combination lies in the fact that PG-600 is the only CG product that can be used in the U.S. for goat reproduction management and even this is an off-label use.

To be successful reproductive management strategies need to use an E/OS protocol structured to permit breeding at the most favorable time in relation to estrus onset in order to increase the opportunity for sperm to fertilize ova. Goats exhibiting natural estrus ovulate 32.5 ± 1.0 h (n = 23) after standing estrus for nulliparous goats, and 36.5 ± 1.1 h (n = 13) for multiparous goats.[163]

Fixed-timed insemination relies on a great majority of synchronized females ovulating at a given time and breeding should take place during an appropriate window to maximize fertilization. However, among the various effects that exogenous hormones can have (*i.e.,*

CG's) is that of modifying ($P<0.05$) the time of ovulation[163] to such an extent that it may interfere with the desired fertilization.

In a study conducted to determine the effects of eCG and PG600 on the timing of sheep estrus and ovulation after progestogen withdrawal, the authors concluded that to prepare ewes for fixed-time AI, eCG was a better choice than PG600 as the gonadotropin to use at the time of progestogen withdrawal.[164]

On the other hand, in a study with lactating goats (≥120 DIM) there was no significant difference between does treated with the hCG/eCG combination and those treated with reagent-grade eCG, in terms of the percentage of does in estrus (89 and 97%, respectively).[165] These same authors found however, a significant difference for pregnancy rate by natural service (90% vs 76% for hCG/eCG and eCG treatment groups, respectively) suggesting the animals are better than managers at determining best time for insemination.

Estrus and ovarian response in both breeding and anestrous season were evaluated using different doses of PG600 (0, 80, 160, 320, 640, and 1,280 IU) at the time of norgestomet withdrawal following 12 d exposure. Estrus behavior and time to estrus were not affected by dose level, but CL number increased with PG600 dosage increases in both seasons.[166]

When ewes of three genotypes in seasonal anestrous were used to determine reproductive performance by using melengestrol acetate (MGA) and/or PG-600 in inducing fertile estrus, ovulation rate of ewes exposed to rams was increased from 1.79 for untreated control ewes to 2.19 for ewes receiving PG600 ($P<0.05$), although the lambing rate was not different ($P>0.05$) between control ewes and ewes treated with PG600, having a

lambing rate of 15% and 18%., respectively.[167] Similar results were obtained using MGA and /or PG600 in Rambouillet ewes.[168]

Decreased RP was evident in a 3-year study using Alpine goats inseminated with frozen-thawed semen in the breeding season following a synchronization protocol based on 18 d P4 PG600.[66] Pregnancy rates decreased from 53% for goats not synchronized and naturally bred to 48% for goats synchronized (no PG600) an inseminated, to 26% for goats synchronized and receiving 600 IU of PG600, 53 48% 26% and 72 to 39.

Despite initial benefits on some measure of reproductive performance, the use of hCG and eCG, separately or in combination, have been shown to have serious drawbacks with goats. Premature CL regression during the early luteal phase has been reported[169, 170] as well as the production of immature follicles[171] and premature chromatin condensation.[172]

Transabdominal ultrasonography for pregnancy diagnosis

The widespread use of ultrasound diagnostic imaging has benefitted reproductive management in farm species in several important ways. Ultrasound imaging (UI) is a non-invasive and rapid procedure capable of providing information about complex internal events offering a means to evaluate pregnancy status earlier in gestation, allowing for improved reproductive management[173] and improved reproductive efficiency.[174]

When managing goat reproduction, UI technology has been used for pregnancy diagnosis, evaluation of embryo number and stage of fetal development.[175-180] Use of UI as a diagnostic tool in female animal reproduction spans a greater possibility of applications in general[181] and for the caprine species in particular. These include early

fetus sexing,[182, 183] fetometry to determine gestational age[184-187 180], transvaginal ultrasound-guided oocyte retrieval,[188, 189] determination of time of ovulation,[163] and assessment of luteal function for embryo donor selection.[190]

Not surprisingly use of UI technology has become widespread and is considered the most efficient diagnostic tool for managing small ruminant reproduction.[191] Nevertheless, the use of UI technology and associated procedures has been widely adopted in the goat based on limited data and insufficient goat group diversity. Although UI has been used in different breeds and animal category groups[192] there is no published information where the specific influence of breed, production phenotype, age and/or parity was considered in terms of potential effect on UI pregnancy diagnosis. Previous UI data generated at our farm was based on its use with more docile milking animals as opposed to more extensively managed, non-tractable, meat and fiber goat phenotypes.[193] Because older goats, regardless of production phenotype or breed, tend to be more accustomed to handling, both age and parity were also of concern because goats used in the previous study ranged from 1.5 to 11 years of age and were of mixed parity.

Real-time B-mode ultrasound quality control testing procedures have been addressed.[194] However, along with equipment quality control, it is necessary to evaluate if the diagnostic test and associated procedure is reliable and consistent.[195]

In this study receiver operating characteristic (ROC) curves[196] were implemented as well as several of the traditional validation metrics were included for validation and evaluation of UI technology under field deployment. ROC analysis is a non-parametric technique largely applied in many fields of medical research and clinical practice.

A ROC curve displays the relationship between the proportion of true positive (sensitivity) and false positive (1-specificity) resulting from each possible decision threshold value in a two-class classification graph, whereas, traditional scalar metrics obtained from a confusion matrix only show one of these possible decision thresholds.[197] For example, in the context of this research sensitivity is the proportion of goat diagnosed pregnant correctly identified as pregnant by the conditions specified to determine pregnancy when using ultrasound imaging (UI). Specificity is the proportion of goats not pregnant correctly identified as "open" by UI. For this reason, (1- specificity) is the proportion of goats that are open but identified incorrectly as pregnant by the screening test. The value of (1- specificity) is referred to as the "false positive rate.[198]

Conveniently, ROC curves provide an area under the curve which can be used as a measure of test accuracy and it permits inferential statistics to be determined between response categories of interest.[199] In this study, results of UI procedures were evaluated independently for the effect of production phenotype and parity on pregnancy status diagnosis and embryo number determination.

Early progeny wastage

Early progeny wastage is regarded as one of the major causes of reproductive failure in the small ruminant livestock industry reaching levels reported to be between 6% and 48%.[200, 201] In a study on the incidence of early embryonic loss in goats based on abattoir specimens there was evidence of embryonic loss in 23.3% of the goats (n=176) and an overall prenatal mortality of 14.9% based on 302 CLs counted.[202]

The consequences of prenatal and perinatal losses are: reduced reproductive performance, slower potential genetic improvement, increased veterinary costs and extension of the generation interval, all of which translate to reduced financial gains. Thus, cost effectiveness of any reproductive program can only be attained if prenatal and perinatal losses are minimized.

Component loss categories of early progeny wastage

Early progeny wastage concerns prenatal (PNL) embryo/fetal survival failure which occur in the period between fertilization and birth at gestation term (i.e., in goats 150 days) and perinatal losses (PL) losses occurring during the birthing process and just after birth. The bulk of the PNL are due to embryonic mortality (EM), abortion and stillbirths.

Prenatal losses associated with embryonic mortality

Due to the complexity of events associated with fertilization and implantation, EM early in pregnancy is usually much higher than fetal losses at later stages of gestation which can be as high a 20 to 30%.[19] In cattle and sheep embryonic and fetal death occurring during pregnancy accounts for 25 to 50% of the total number of fertilized ova.[203, 204] In general, losses due to early EM are difficult to assess under field conditions.[205, 206] Evaluation of early progeny losses of EM fail to distinguish between fertilization failure and actual embryonic death.[207] This is particularly true for tracking actual embryonic death in cases where there has been more than one ovulation and fertilization since mortality may be partial (i.e., only one embryo survives from a batch of two or more embryos); when there is partial litter mortality EM goes largely unreported or is underestimated which effectively introduces a downward bias to EM estimates and, therefore, PNL.[208]

Economic impact of prenatal losses. In general the economic impact of PNL in goats is inadequately quantified largely because there is no information published of its extent. Within PNL, EM represents a large portion of the economic losses. It has been estimated that the cost impact of the EM of sheep and goats to Virginia producers is approximately $1.2 M per year in unattained profits.[209]

Causes for pre natal losses (PNL). The caprine species shares many similarities with sheep and cattle during early portions of embryo development. A large portion of EM may be associated with the CL-endometrium-embryo interactions.[210] In cows it is estimated that approximately 35% of embryos fail to prevent luteolysis during the first three weeks of gestation.[211]

Abortion in goats has been associated with stress and, in turn, with increased maternal cortisol.[212] A portion of the PNL, particularly losses associated with EM occurring under normal and stress-free conditions, are deemed to be unavoidable and are called "basal EM". In fact some of the early postulates declared that basal embryonic death may be a "way of eliminating unfit genotypes at low biological cost".[213] Nevertheless, it is now generally accepted, that identifiable factors exist which cause embryo death rate to rise beyond this inadequately defined or quantitatively determined threshold basal limit.

This study does not address the causative agents of early progeny wastage but the etiology is known to be multifactorial and includes ovulation rate, litter size, endocrine insufficiency (early luteal regression), stress, parasitism, management, infectious agents, disease leading to fever, poor gamete development, inadequate fertilization, high temperature (climatic and body), weight loss and/or poor body condition, nutritional

deficiencies, genetic abnormalities, including the use of assisted reproductive technologies (ARTs).[214]

In an epidemiological study with dairy goats it was found that advanced maternal age, difficulty in conceiving, low social status, pregnancy with at least 3 fetuses and previous fetal loss were significantly associated with current loss.[215]

Other factors may also be of importance depending on the type of management production setup involved, such as, death of a pregnant mother, pre-term cesarean interventions and perinatal losses, circulating concentration of P4 during the early luteal phase of the cycle following insemination.[216] Some kid mortality which occurs closely after birth (PL) may be associated with and/or are a consequence of undetermined prenatal events.

The incidence of goat prenatal wastage in the U.S. has not been addressed satisfactorily. All the same, preliminary data collected at Lincoln University, Jefferson, MO by Wurst and Rathert (2009)[217] utilizing real-time transrectal and transabdominal ultrasonography revealed that 23% of does pregnant had either partial or total PNL and that on sequential years 76 and 95% of the losses occurred after day 60. If the timing of these losses are characteristic of the caprine species that would mean they are not similar to patterns found in sheep, where losses occur across gestation, or akin to cows, where 47% of the recorded losses occurred between days 28 and 42 of gestation.[216]

Time-line and quantitative description of prenatal loss. As stated previously, the methodology used to determine and quantitatively describe PNL has largely been the obvious sign of a female not showing characteristic behavior of sexual receptivity on her

next scheduled estrus, 18 to 24 days after breeding, but rather as late as 35-50 days after insemination. Does have been observed to have a normal 21-day estrous cycle if no fertilization occurs or if the embryos die before day 15, when maternal recognition of pregnancy occurs[37]. For this reason, EM occurring before maternal recognition of pregnancy cannot be discriminated from cases of unsuccessful fertilization. More recent research has used hormonal analysis and ultrasound technology to follow CL and embryo dynamics with the limitation that the earliest embryos can be detected using blood plasma P4 analysis is day 20 post insemination and day 30 by means of transrectal sonography or day 40 using transabdominal sonography.

Role of chorionic gonadotropins in conception and embryo survival

Low conception rates and enhanced embryo mortality have been associated with tardy increase in systemic P4 concentration. hCG increases P4 levels[218, 219] and increased levels of P4 have been shown to be beneficial to embryo viability.[220-222] The practical significance of using hCG is to stimulate luteal function and increase P4 production.[223] Improved conception rates using hCG on days 5 to 7 after AI in lactating goats have been reported.[218]

Khan and colleagues, (2003)[224] determined that the total number of lambs born (saline: 38; hCG: 58) was significantly ($P<0.05$) greater in the hCG-treated group compared to the saline-treated controls. Lambing percentage (saline: 36%; hCG: 48%) and litter size (saline: 1.35; hCG: 1.48) tended to be greater ($P<0.10$) in hCG-treated animals compared to the controls. These results may be a consequence of improved embryo viability, which was also demonstrated by the previous authors.[224] hCG provided at time of mating was shown to significantly ($P<0.05$) increased crown-rump length from 11.9±0.2 mm for the

saline/control group to 12.7±0.2 mm for the hCG-treated group, amniotic sac width (saline: 11.4±0.4 mm; hCG: 12.0±0.3 mm) and the number of placentomes (saline: 90.8±7.3; hCG=122.4±6.3).

Uterine cervix characterization and response to E/OS protocols

Reproductive management of goats has followed the inroads advanced by other species, mainly the bovine and the ovine, but in general has lagged behind the existing knowledge for other farm animals. When AI was adopted the goat industry also adopted many of the methodologies used to breed cows and sheep. In both sheep and goats, conception rates, pregnancy rates and kidding rates are quite variable and often pregnancy rates following first service are reported at below 40%,[216]

One of the long-lasting tenets of AI biotechnology is the affirmation that when using frozen-thawed semen, greater fertility is obtained the further semen is deposited in the reproductive tract closer to the site of natural fertilization.[225, 226] Most contemporary recommendations of trans-cervical AI suggest semen should be deposited in the cervix body[227, 228] or in individual uterine cornua of cows[229] and goats.[230] In cows no difference was found between semen deposition in the uterine body or uterine horns.[231 232]

Yet some research in goats has not yielded differences related to AI depth of insemination[233] and or in fact have seen the opposite results.[234] Good results were attained by farmers[235] placing thawed frozen semen in the vagina of goats with non-return rates (NRR) and kidding rates after single insemination of 64.3% and 58.3%, respectively. Or, when using double inseminations, the result was a NRR of 62% and a kidding rate of 57%.[235] In sheep there was no significant difference (P=0.40) between

kidding rate resulting from cervical semen deposition (78%)and vaginal inseminations (74%).[236]

Fertilization in ruminants takes place in the ampullary region of the oviduct soon after ovulation.[237, 238] It is accepted that fertility rates, in the bovine, ovine and caprine, are close to 90 to 100%,[216, 239-242] In spite of the high fertility rates documented, from a practical point of view, the uterine cervix, is the anatomical region which, because of its convoluted nature, poses the greatest challenge at time of artificial insemination. Aside from morphological and biometrical studies,[243-246] the role of the cervix in limitations of transcervical AI in small ruminants has been poorly taken into account.

Gross anatomical and histomorphological changes

The non-pregnant healthy cervix in the goat contains approximately five fibrous overlapping internal tissue flaps (annular folds) commonly referred to as "rings". The cervix normally varies in length with breed, age, season and stage of the estrous cycle. On average ± SD the cervix measures 5.73 ±0.35 cm in length and 1.60 ±0.19 cm in breadth.[247] The gross anatomy of the cervix (*i.e.,* os cervix and annular folds) determines the ease of inseminating pipette passage and hence the success of transcervical AI. The anatomy of the goat cervix has not been evaluated in a large number of females, however, the sheep cervix has been found to be highly variable between animals.[248]

In comparative anatomical studies Bunch and Ellsworth, (1981)[249] reported that caprine cervices were less tortuous than their ovine counterpart with more concentric alignment in their annular folds where characteristically the second annular fold opening is found eccentric to the first fold interfering with normal passage of the AI pipette. The aforementioned authors[248] quantified the variation in cervical morphology between ewes

and have established the relationship between cervical anatomy and cervical penetration. These previous authors classified the morphology of the cervical external os as: slit, papilla, duckbill, flap or rose. Maximum depth of cervical penetration with an insemination pipette was affected by cervical grade (one of three anatomical configurations related to degree of cervical lumen convolution) and the stage of the estrous cycle. The distribution of os types differed with age.[248] These observations were confirmed in that prepubertal goats (4 and 8 months old) induced to estrus revealed a significant increase in the length and width of the cervix.[250]

Oxytocin is one of the important pituitary hormones known to have dramatic effects over cervical physiology both at estrus and parturition. At estrus its presence triggers relaxation of the cervix and at parturition it contracts the uterus. It is surprising that the cervix should be insensitive to large doses of oxytocin whereas adrenaline relaxes the cornea and causes the cervix to contract.[251] More recent information conveys a different picture where oxytocin in the ovine cervix at estrus is coincident with increases in oxytocin receptor expression and electromyographic activity responsiveness.[252]

Indeed, changes in the goat cervix can be observed at time of estrus when the cervix structure relaxes (presumably under the influence of estrogen) and, since the cervical lumen is lined with pseudo-stratified columnar epithelium in the form of Goblet cells[250] the cervical mucus becomes more profuse and liquefies releasing the characteristic mucus secretion during estrus that in the internal reproductive tract serves as the medium where sperm advanced towards the fertilization site. The secretion also serves as a convenient natural lubricant that facilitates TrAI.

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CHAPTER III

ASSESSMENT OF DIAGNOSTIC VALIDITY OF PREGNANCY DIAGNOSIS AND FETUS NUMBER DETERMINATION BY ULTRASOUND IMAGING IN MIXED PARITY DAIRY, MEAT AND MEAT/FIBER GOATS

Abstract

Despite extensive use, real-time goat ultrasound imaging (UI) technology requires validation under a wider scenario of representative production phenotypes, ages and environmental influences. This study used UI for pregnancy and fetus number diagnosis in dairy, meat and meat/fiber production phenotypes of different parities. Transabdominal UI was performed at 45 ± 4 d post breeding (n=448; 1.5 to 10 y old). UI validation metrics used were: sensitivity, specificity, precision, accuracy, false-positive rate, and negative predictive value. The relationship between sensitivity and false-positive rate was determined by receiving operating curve analysis. Odds ratios (OR) were used to establish the influence of production phenotype and parity on the various UI metrics. A Spearman's rank correlation coefficient (ρ) of 0.91 (P<0.0001) was found between pregnancy diagnosis and parturition outcome and, a ρ of 0.84 (P<0.0001) between fetus

number estimation and litter size. UI diagnosed correctly 96% of non-pregnant goats, 85% of twins, 80% of singletons and 79% of triplets. All diagnoses were different ($P<0.0001$) from no effect at 50%. Pregnancy diagnosis had greater precision (100%) for dairy ($P<0.027$) than for the meat/fiber production phenotype (91.7%). Fetus number precision was influenced by production phenotype (OR= 8×; $P<0.02$) and parity (OR= 2×; $P<0.03$). Accuracy was compromised due to parity OR= 2×; ($P<0.01$). In conclusion, UI data of different goat production phenotypes and parities can be used to determine pregnancy status; however UI data generated for fetus number in dairy and meat production phenotypes as well as for multiparous and primiparous goats requires further confirmation.

Introduction

Ultrasound imaging (UI) is a non-invasive and rapid procedure capable of providing information about complex internal events and possibly offering a means to evaluate pregnancy status earlier in gestation,[1, 2] allowing for improved reproductive management[3, 4] and improved reproductive efficiency.[5] In spite of limited field data, use of UI technology has become widespread and is considered the most efficient diagnostic tool for managing small ruminant reproduction.[6, 7] In the caprine species UI has been used primarily for pregnancy diagnosis, evaluation of fetus number and stage of fetal development.[8-14]

Although UI has been used in different breeds and animal category groups[15-19] there is no published information where the specific influence of production phenotype, age and/or parity has been considered in terms of potential effect on UI pregnancy diagnosis. Previous UI data generated at our farm[20] used the more tame milking animals as opposed to more extensively managed goat breeds. We hypothesize that variability in UI test results may be due to the difference in ease/difficulty of handling animals. In the context of this study, intractable goats correspond to less docile groups; phenotypically corresponding to extensively managed non-dairy animals (meat and meat/fiber herds) and the less cooperative nulliparous age category.

Real-time B-mode ultrasound basic principles, terminology and the application of this technology to ruminants have been discussed previously. [7, 21-23] Besides creating superior images, it is necessary to evaluate if the diagnostic test and associated procedure used to collect the necessary information is reliable and consistent.[24-26]

In this study receiver operating characteristic (ROC) curves[27-30] were chosen to analyze the diagnostic data generated by the UI because they provide useful information which is easy to grasp visually as well as providing a robust means to test modeled differences between UI scans. ROC analysis is a statistical procedure with a long history in medical diagnostics[31] and has been used extensively in various areas of obstetrics in conjunction with results obtained through the use of radiology and ultrasonography.[27] Although not as frequently used in clinical theriogenology, at present ROC analysis has been widely accepted as the standard for describing and comparing the accuracy of diagnostic tests.[32] Additionally, we provide standard validation metrics for UI field evaluation comparison. Results of UI procedures were studied independently for the effect of production phenotype and parity category on reproductive status and fetus number determination.

Materials and Methods

This study was conducted under field and research facility conditions using guidelines for animal care and use at the American Institute for Goat Research (AIGR), Langston Oklahoma (*Lat*. 35.945° N *Long.* -97.255° W; 292 meters above sea level). Daylight hours ranged from 12.8 h to 9.6 h from the beginning to the end of the breeding season.

Goats used in this study ranged in age from 1.5 to 10 y (mean ±SD = 4.1 ±1.5 y). Goats were randomly assigned to three different breeding procedures (i.e., natural service, transcervical and laparoscopic-aided intrauterine artificial insemination) during the 2007 and 2008 breeding seasons. Breeding procedures were not expected to influence the outcome of UI results because the latter is a diagnostic tool that records the outcome of a separate time/space independent reproductive event of pregnancy or its absence. Goats of 5 breeds (i.e., Alpine, Angora, Boer, Spanish and Tennessee Stiff-Leg) and various genotypic percentage crossbreds were grouped according to representative production phenotypes: dairy, fiber/meat and meat. Goats were also grouped according to parity: nulliparous, primiparous and multiparous.

Animal Management

The Alpine herd consisted of non-lactating goats managed semi-extensively on Bermuda or Sudan grass. The meat and meat/fiber herd was managed extensively on native Oklahoma mixed grasses. Both herds had access to wheat pasture when available. Diets were supplemented with either a low or a high protein commercial custom-manufactured goat pellet supplement (Stillwater Milling Co.; Stillwater, OK) with 13.3 and 20.3% crude protein, respectively. Ultrasound scanning was done in two indoor facilities.

All goats received fresh water, free access to mineral supplement, and access to shelters. Goats were cared for and monitored daily by farm personnel. All goats were under veterinary care and treated regularly for internal parasites with anthelmintics (i.e., Cydectin® (Fort Dodge Animal Health, Fort Dodge, IA) or Valbazen® (Pfizer Animal Health, Exton, PA) or Levazole® (Schering-Plough Animal Health, Summit, NJ).

Sample size by production phenotype and parity categories

A total of 448 goats were used. Production phenotypes were: dairy (n= 87), fiber/meat (n= 20) and meat (n= 341). Goats were also grouped according to parity: nulliparous (n= 126), primiparous (n=195) and multiparous (n=127).

In the context of this study, intractable goats were goats difficult to perform an UI scan. Intractable goats were represented by the least docile categories phenotypically corresponding to the extensively managed meat and fiber herds -i.e., 361/448= 81% and the nulliparous doelings (3.1 ±0.69 y) –i.e., 126/448= 28 %.

Pregnancy diagnosis and evaluation of the predicted number of fetuses

Predicted reproductive status (pregnant or open) and fetus number were evaluated in accordance to established procedures developed previously for Alpine goats.[20] Briefly, pregnancy diagnosis was based on the recognition of any or a combination of the following: fluid filled uterine horns, the presence of placentomes, fetal structures (e.g. head, thorax, limbs, beating heart) and fetal body movements if observed.

Pregnancy diagnosis was targeted to be done at approximately 45 days post breeding (dPB). The final call regarding reproductive status was the sole responsibility of one technician throughout the research project. Unlike a previous UI study done with sheep[33]

our work did not consider an evaluation on the degree of certainty in the reproductive status diagnosis. We feel that the results our team obtains are equivalent to results obtained by the rest of the industry.

UI scanning was performed by mid ventral external examination of a pre-clipped area using a portable ALOKA SSD-500V (Aloka Co. Ltd., Japan) equipped with a 3.5 MHz linear array transducer (UST-934N) mounted on an external scanning device. To increase contact of the transducer with the animal's body the observation area skin surface was sprayed with alcohol.

Evaluation of diagnostic performance

A confusion matrix (contingency table) customarily used to organize UI metrics was generated to evaluate diagnostic performance (Appendix A). In this study UI metrics are defined as statistical measures of the performance of a binary classification test (*i.e.,* reproductive status and fetal counts), also known in statistics as a classification function for evaluating diagnostic performance.

Results of pregnancy determination by UI scanning were confirmed to actual fertilization date at the end of the kidding season by backtracking 150 ±10 days from actual parturition day. Likewise, the number of fetuses detected at time of UI scanning was compared to the actual litter size obtained at term. Hence, true positive and true negative diagnostic outcome values were recorded when predicted reproductive status (i.e., pregnant or non-pregnant) or predicted fetus number, based on UI at ±45 d post breeding, matched the results at parturition for each individual goat. All false/positive outcomes resulted from observation where: 1) A doe was determined to be pregnant by UI at ±45 dPB and failed to give birth at putative gestation term, or 2) A given number (n) of

fetuses were recorded on the basis of UI at ±45 dPB and there was a discrepancy with the actual litter size of n-1 or n+1 at parturition. Conversely, all false/negative outcomes resulted from observation where: 1) A doe or doeling was determined not to be pregnant by UI at ±45 dPB but gave birth at term, or 2) A given number (n) of fetuses were recorded (including none or zero) on the basis of UI at ±45 dPB and there was a discrepancy with the actual litter size at parturition where the actual n was greater or smaller than the predicted n.

Data analysis

Computerized statistical analyses

Unless noted in the text, all data was analyzed with and corresponding graphs were obtained using the computerized statistical analysis system JMP v.9.[34]

Number of ultrasound scans

There were a total 448 UI scans. Of these, 7 does that kidded and had litter sizes of 4 and 5 kids were excluded from statistical analyses involving the number of fetuses only, due to insufficient sample size per category to be representative of a given fetus number group. The remaining 441 observations were included in the analysis.

Validation of ultrasound imaging procedure

Use of UI for pregnancy detection at ±45 dPB was evaluated by comparing diagnostic values generated by the UI scans against actual parturition data using the Pearson's correlation and confirmed with the non-parametric Spearman's rank correlation coefficient. Both statistics have been described previously.[46]

Cohen's agreement statistic (κ) was used to match levels across two categorical variables[35, 36] as well as by using Bowker's test for symmetry.[37, 38] Additionally, calculation of relative error rates and likelihood ratios were performed in accordance to previous recommendation.[28, 39,40] The proportion of goats with positive UI test results (e.g., pregnant) or negative results (e.g., non-pregnant) that were correctly diagnosed, that is the positive or negative predictive values, respectively, were determined as has already been suggested.[41]

The relationship between the true positive rate and the false positive rate resulting from UI pregnancy diagnosis and fetus number evaluation was determined using receiver operating characteristic (ROC) plots as described for other studies.[42-45]

Ultrasound imaging metrics used to evaluate diagnostic outcome

UI validation statistics and relationships among metric terms were determined from the ±45 dPB UI scanning results which were validated by confirmation of kidding outcome at term. The birth of at least one kid confirmed UI scans of positive pregnancy. Actual litter size confirmed UI evaluation for fetus number.

UI metrics are used in the context of a classical confusion matrix as originally proposed in the context of artificial intelligence.[47] That is, metrics chosen to evaluate diagnostic outcome were: sensitivity, specificity, accuracy, precision and the positive and negative predictive value of UI detection for pregnancy and fetus number diagnosis was determined according to already established formulae [43, 48] as follows:

Sensitivity. Represents the true positive rate (TPR) and is equivalent with "hit rate" or recall.

$$\boldsymbol{TPR} = TP / P = TP / (TP + FN)$$

where,

TP represents the true positive values and P are all the positive values. That is, all the correctly declared positive while FN represents all the false negative values.

Specificity (SPC). Describes the true negative rate; equivalent to the correct rejection rate.

$$\boldsymbol{SPC} = TN / N = TN / (FP + TN) = 1 - FPR$$

where,

TN represents the true negative values, N represents all the negative values (true and false negative values), FP are the false positive values, and FPR represents the false positive rate.

Accuracy (ACC). Accuracy is the proportion of true results (both true positives and true negatives) in the population.

$$\boldsymbol{ACC} = (TP + TN) / (P + N) \quad \text{or} \quad \boldsymbol{ACC} = (TP + TN) / (TP + FP + TN + FN)$$

where,

TP represents all the positive values (true and false positive values), TN represent the true negative values, P are all the positive values (true and false positive values), N represents all the negative values (true and false negative values), and FN represents all the false negative values.

Precision. Precision is defined as the proportion of the true positives against all the positive results (both true positives and false positives). It is calculated by the positive predictive value (PPV).

$$\boldsymbol{PPV} = TP / (TP + FP)$$

Where,

TP represents all the positive values (true and false positive values) and FP are the false positive values (true and false negative values).

False negative rate (FNR). Represents all the "misses" and is equivalent to the statistical inferential Type II error, i.e., failure rejecting a false null hypothesis. FNR can also be calculated from 1 – sensitivity.

$$\boldsymbol{FNR} = FN / (TP + FN)$$

Where,

TP represents all the positive values (true and false positive values) and FN represents all the false negative values.

False positive rate (FPR). Characterizes what is commonly known as "false alarm" or the fall-out rate and in statistical theory it represents an inferential Type I error, i.e., when a true null hypothesis is incorrectly rejected.

$$\boldsymbol{FPR} = FP / N = FP / (FP + TN)$$

where,

FP represents the false positive values, N represents all the negative values, (those regarded as true when actually false), and TN represents all true negative observations.

Percent relative error rate (%RER). The percent relative error rate was calculated using the following formula:

$$\%\boldsymbol{RER} = \frac{|\text{Absolute error}|}{\text{True value}} \times 100$$

where,

the absolute error is the absolute difference between the calculated and the true value. The error rate was determined on the basis of probabilities generated by data fitted to a nominal logistic model: response variable (i.e., pregnancy diagnosis or fetus number evaluation) = classification variable (i.e., goats that kidded or litter size, respectively) as established.[49] Where the most likely predicted probability is determined by choosing which probability, from either P [Pregnant |x] or P[Open |x]), turns out to be greater. A similar strategy was implemented for the evaluation of fetus number diagnosis at 4 levels of prediction (none, 1, 2 or 3 fetuses detected). A contingency table was created for each comparison (see Table 3 and 4) where the most likely high [Pregnancy Adjusted] is the independent variable (X) and the high [Pregnancy Adjusted] is the response variable (Y).

Receiver operating characteristic (ROC)

In this study ROC curves are used to measure the sorting efficiency of the model's fitted probabilities to the response levels (pregnancy status or fetus number). A criterion value (c) for UI diagnostic tests will also be produced on the basis of maximizing the total area under the curve (AUC).

Criterion values, likelihood ratios and predictive values of the ROC curve. Criterion values as well as the likelihood ratios and predictive values of the ROC curve were determined using the statistical biomedical analysis system.[50]

Results

Assessment of pregnancy diagnosis and fetus number detection methodology

Real-time (B- Mode) ultrasonography technology was used in this study (n=448) for diagnosing reproductive status (i.e., pregnant or non-pregnant) and for estimating the number of fetuses per pregnancy (n=441) at a target date of ±45 dPB. Five goat breeds were represented: Alpine, Angora, Boer, Spanish, and Tennessee Stiff-Legs (different percentage cross-bred genotypes were also used) with a mean age (±SD) of 4.1 ±1.5 years.

Pregnancy rate

As presented in Table 1, a total of 246 goats (55%) were diagnosed pregnant and 202 (45%) were reported open by ±45 d UI. These percentage results turned out to be the same as the percentages obtained at term for goats kidding and goats open to the first breeding, correspondingly.

A summary of the overall average and variability (±SE) of the UI metrics selected to depict pregnancy diagnosis effectiveness are shown in Table 1. Results were calculated by evaluating data values from the UI diagnosis and corresponding parturition results. We found sensitivity (percentage of true positives) of 95.2 ±0.8%, a specificity (percentage of true negatives) of 94.5 ±1.7%, precision was 95.5 ±1.4%, accuracy (summary of correct results) was 94.8 ±1.1%, the false positive rate (type I error) was 5.5 ±1.7%, and the negative predictive value was 93.3 ±1.3%.

Number of fetuses

A summary of the overall results obtained concerning the number of estimated and actual litter size obtained at parturition is shown in Table 2. Although 201goats were diagnosed open and 201did not kid at term, these did not necessarily correspond to the same 201 animals. The number of diagnosed and kidded sets of twins was 33% (n= 149) and 29% (n=132), respectively. Singletons were diagnosed 18% of the time (n= 82) and 15% (n=69) actually gave birth to one kid at term. The greatest discrepancy was with litter sizes of triplets, quadruplets and quintuples, where only 4% (16 goats) were diagnosed as pregnant with more than two fetuses, but the number of females giving birth to more than two fetuses was 10% (n=46) of all goats bred or 19% of all pregnant goats (n=247).

Taken together the low values encountered for UI evaluation metrics i.e., sensitivity and the negative predictive value were 93%, specificity and precision were below 70%, and accuracy was 76% describes a scenario where UI as a diagnostic tool under the conditions of this study is unreliable. Likewise a type I error 33.5% (see Table 2) implies that the false positive rate is so large that the probability of finding statistically significant differences between to UI diagnosed groups is very unlikely. The wide range of variability recorded in the UI metrics, where the least and the most variable were sensitivity with ±0.8% and precision with a ±6.4%, respectively presuppose that for research purposes each variable would require a different sample size to detect a given difference between treatments.

Correlation analysis

Pregnancy diagnosis

UI pregnancy status and kidding at term yielded a correlation coefficient (r) of 0.91 with $CI_{95\%}$= (0.887, 0.921). This correlation was confirmed with the non-parametric Spearman's rank correlation ρ coefficient of 0.91 ($P<0.0001$) associated with the same comparison indicating strong overall association between UI diagnosis of pregnancy at ±45 d PB with pregnancy resulting in live births at term; ±150 days.

Estimated fetus number

Likewise, an r of 0.80 with $CI_{95\%}$= (0.766, 0.833) confirmed with a Spearman's rank correlation (ρ) value of 0.84 ($P<0.001$) was obtained for the estimated fetus number and while numerically 16 percent units below the association found between UI diagnosis for pregnancy and kidding at term the r coefficient indicates an important overall association.

Symmetry of agreement and error rate of ultrasound imaging

Pregnancy diagnosis

Further analysis resulted in no difference ($P>0.05$) being found between UI scans for pregnancy diagnosis and actual kidding results. The symmetry of agreement (statistic κ) for pregnancy diagnosis was calculated to be 0.91 ± 0.02 SE with a $CI_{95\%}$= (0.8657, 0.9448). This means that κ, the difference between actual agreement, and agreement expected by chance compared to (divided by) the scope for doing better than by chance is very high and that the strength of agreement between UI at ±45 dPB (n= 448) and the resulting actual pregnancies, as determined by does that kidded, was very good according to a standardized scale.[51]

The Bowker's test value was 0.05 (P>0.82). This result confirms the previous κ statistic results, by failing to find sufficient statistical evidence to reject the null hypothesis that the probabilities calculated satisfy symmetry. That is, that UI results are essentially equivalent to what one observes at kidding time.

As presented on Table 3, the overall relative error rate (%RER) for UI pregnancy detection at ±45 dPB was (21/448) ×100= 4.7%, with an even distribution between the %RER for non-pregnant and pregnant goats. That is, (10/246) ×100= 4.1% RER was associated with open goats and (11/202) ×100= 5.5% RER when pregnant.

Similarly, 4.1% of goats deemed to be open were in fact pregnant. The significance of this value can be better evaluated if comparison is made with events connected to routine behavioral estrus, where approximately 12% of pregnant goats express estrus in their upcoming next scheduled estrus (as observed in our herds; data not published). The estimated overall relative error rate of 4.7% is considered low.

Estimated fetus number

The symmetry of agreement (statistic κ) for fetus number evaluation by UI at ±45 dPB (n= 441) was calculated to be 0.61 ± 0.03 SE with a $CI_{95\%}$= (0.5542, 0.6658) (P<0.0001). Although a κ value of 61% would classify UI for fetus number detection as good,[51] a significant difference (P<0.02) was found when UI was used to predict litter size at kidding time by the number of fetuses scanned at ±45dPB.

The Bowker's test value for symmetry of disagreement for fetus number was 22.8 ($P<0.0009$) and confirms the preceding result. That is, taken together, both κ statistic and Bowker's test, suggest that UI at ±45 dPB is an unreliable predictor of litter size at birth.

Once the anticipated status for the presence/absence of a particular number of fetuses was established, the resulting most-likely predicted value was compared to actual pregnancy status as diagnosed by UI scanning.

A total RER of 114/441= 25.9% was calculated (Table 4). RER's distributed among different fetus number diagnosed were: 5.5%, 58.3%, 34.3% and 38.5% for 0, 1, 2 and 3 predicted litter size, respectively. This implies that 26% of the relative error calculated for the goats in this study has to do with determination of litter sizes of at least one fetus.

Clearly the large RER (26%) for fetus number evaluation ($P<0.0001$) reported is a consequence of the lack of correspondence between predicted fetal number and realized litter size at term. As shown, this discrepancy was also confirmed by Bowker's test value for the symmetry of disagreement. Relative error rates when evaluating the number of fetuses present at ±45 dPB (n=441) showed that 4.5% of the does determined to be open were carrying a singleton fetus and 1% carried twins.

As given in Table 4 (junction of column labeled "1" and row labeled "0") 7.25% of does determined to be carrying a single fetus actually were open and 42% had twins. Less than 4% of the does detected with two fetuses were not pregnant, 21% carried 1 and 4%had triplets. Of the does diagnosed with 3 fetuses 2.6% were not pregnant, 31% had 1 fetus and 46% had twins. In summary since 35 goats were correctly diagnosed as carrying 1 fetus out of 69 (50.7%) the level of misdiagnosis for goats carrying 1 fetus was 49.3%.

Receiver operating characteristic (ROC) analysis of UI diagnostic performance

Reproductive status

The relationship between sensitivity and the false positive rate (1-specificity) for pregnancy status diagnosis was analyzed by ROC analysis. Sensitivity represents the true positive rate and is equivalent with "hit rate" or recall; specificity describes the true negative rate; equivalent to the correct rejection rate. The nature of the relationship was that of a direct and positive association. Based on the nominal logistic model previously described (n=448), the selected area beneath the ROC curve was calculated to be 95% ±0.01SE which was different ($P<0.001$) than the 50% random distribution of no effect as shown in Figure 1. As can be seen in Table 5, using the selected criterion value (*) the 96% sensitivity was determined to have a $CI_{95\%}$= (0.92, 0.98) and a specificity value which reached 95% with a $CI_{95\%}$= (0.91, 0.98) (Table 5). These statistics show that both sensitivity and specificity when using UI at ±45 dPB to determine pregnancy is very reliable across production phenotypes and parity categories.[48]

Number of fetuses

ROC analysis for predicted litter size at kidding by UI diagnosis at ±45 dPB, resulted in all curves placed above the reference curve of no-efficiency at the 45º diagonal ($P<0.001$) (Figure 2). This is interpreted that having rejected the hypothesis that the theoretical area is 0.5 (*i.e.,* unable to discern between number of fetuses) we conclude there is evidence that the diagnostic test does have the ability to distinguish between the four groups.

An UI scan interpretation of the AUC under the scenario that the goat is not pregnant (no fetus detected) means that a randomly selected doe, from the group where UI diagnosis

has been that of a no pregnancy, will not kid 96% of the time compared with a randomly chosen doe from the pregnant group (with 1, 2 or 3 fetuses). Followed by the ability to determine twin fetuses at 85%, followed by the ability to diagnose the presence of one fetus at 80% and finally, triplets were detected correctly with a 79% of the time.

Predictive values and likelihood ratios

Precision is the proportion of the true positives against all the positive results (both true positives and false positives), calculated by the positive predictive value (PPV). Accuracy is the proportion of true results (both true positives and true negatives) in the population. The false positive rate (FPR) represents the type I error (α value). Negative predictive value (NPV) is the proportion of the true negatives against all the negative results (both true negatives and false negatives).

A likelihood ratio represents an expression of probability of test results, given the presence (and absence) of a given condition.[52] This conceptual meaning is interpreted in the context of this study as the ratio between the probability of a defined UI test result (i.e., pregnant or not pregnant and/or the number of fetuses) given that a diagnosis has already been advanced (presence of the condition) and the probability of a defined test result given the absence of the condition.

The positive predictive value or precision of a test is the probability that a given condition exists when the observations are restricted to those observations that test positive.[42] The positive likelihood ratio (+LR) and the precision (positive predictive value) were: 19.2 and 95.6 with $CI_{95\%}$ of (18.4, 20.0) and (92.7, 98.0); respectively (Table 6). This means that pregnancy was about 19 times as likely to occur in a goat diagnosed

by UI at ±45 dPB as being pregnant as in a goat whose UI diagnosis resulted in no pregnancy. Based on UI at ±45 dPB the probability of a goat being pregnant was of 96% when only goats diagnosed as pregnant were considered in the calculation.

Likewise, the negative likelihood ratio (-LR) and negative predictive value were: 0.1 (1/10) and 94.6 with $CI_{95\%}$of (0.02, 0.10) and (90.5, 97.3); respectively (Table 6). This means that the condition of a goat not being pregnant was very small (about 1 in 10) times as likely to occur in a goat diagnosed by UI at ± 45 dPB as being pregnant as in a goat whose UI diagnosis resulted in no pregnancy. Based on UI at ±45 dPB the probability of a goat not being pregnant was of 95% when only goats diagnosed open were considered in the calculation.

Ultrasound imaging diagnostic metrics

Ultrasound metrics for pregnancy status

The overall UI diagnostic metrics for pregnancy status not considering production phenotype and/or parity subgroups had values at approximately 95% with a 5.5% type I error (Table 7). The average of all ultrasound evaluation metrics were close to 95% except for the NPV which was recorded as 93%.

Statistical comparisons for UI pregnancy status according to production phenotype.

Comparisons between production phenotypes (Table 8) did not yield significant differences for sensitivity with P values ranging from (P>0.681) to (P>0.428). Dairy and fiber production phenotypes had specificities of 100 and 87.5, respectively (P<0.063). Other comparisons for specificity were not significant (P>0.215). Precision was significantly greater (P<0.027) for the dairy production phenotype (100%) compared with

the fiber production phenotype (91.7%). The odds ratio (OR) for this latter comparison could not be calculated because of the presence of an empty cell in the OR calculating matrix. Other comparisons between production phenotypes did not influence precision of UI scanning (P>0.079) for pregnancy diagnosis. Production phenotype had no influence on accuracy of UI evaluations (P>0.101 to P>0.329).

Ultrasound metrics for pregnancy status according to goat parity. All the metrics evaluated were close to 95% or greater and the type I error rate was under 5%. As can be appreciated in Table 9, parity grouping (i.e., nulliparous, primiparous and multiparous) was the only independent variable studied that had no influence over any of the metrics used to evaluate UI scanning for the purpose of pregnancy diagnosis. Sensitivity was not influenced by parity categories and ranged from P>0.616 to P>0.832. Parity had no influence over the specificity metric with a range of P values from P>0.353 to P>0.950. Parity was non-influential over the precision metric with a range of P values from P>0.092 to P>0.939. Finally, parity did not influence accuracy which yielded a range of P values from P>0.544 to P>0.770.

Ultrasound metrics for fetus number determination

Ultrasound diagnostic metrics for fetus number determination, not considering production phenotype and/or parity subgroups yielded overall lower values than those obtained for pregnancy status diagnosis (Table 10). Sensitivity was 93% and specificity and accuracy were 67% and 76% accordingly. Type I error was 33.5%. Variation among UI metrics for fetus number determinations was numerically much greater (range of 0.8 to 6.4 ±SE) than that found for the same metrics when diagnosing pregnancy status, which ranged from

0.8 to 1.7 ±SE. The false positive rate or type I error, with a desired expected value below 5%, was calculated to be on the average 34%.

Statistical comparisons for UI evaluation of fetus number according to production phenotype. Comparisons between production phenotypes (Table 11) did not yield any significant differences for sensitivity with P values ranging from P> 0.666 to P>0.996. Specificity was statistically close to significance between dairy (51.9%) and fiber (87.5%) production phenotypes at (P<0.059) and (P<0.077), respectively. UI performed on fiber goats had a precision of 92% and differed from meat (52.5%; P<0.01) and from dairy (56.9%; P<0.02) production phenotypes with OR's of 10× and 8×, respectively. Other production phenotypic comparisons did not influence accuracy with P values ranging from (P>0.092) and (P>0.451).

Ultrasound metrics for the evaluation of fetus number according to goat parity. The metrics evaluated were as variable as found when evaluating the effect of production phenotype on UI evaluation metrics. As described in Table 12, type I error rate was at its highest value with 35.1 ± 4.9%. Comparisons between parity categories did not yield any significant differences for sensitivity with P values ranging from P>0.384 to P>0.841. Nulliparous and primiparous goats had specificities of 72.4% and 55.6%, respectively (P<0.012). Other comparisons between parity groups for the specificity metric were not significantly different (P>0.111).

A significant difference (P<0.028) between multiparous (63.9%) and primiparous goats (47.4%) was found with respect to the precision metric. Other production phenotypes did not influence the precision of UI scanning (P>0.094). Parity also influenced accuracy of

ultrasound scanning (P<0.035) between multiparous (77.2%) and primiparous (66.2%) goats and between nulliparous (79.4%) and primiparous goats (66.2%); (P<0.011).

UI performance according to ROC analysis

Many of the UI metrics generated in a confusion matrix are used to statistically compare different independent groups in an experimental setting. The treatment difference expressed as percentages can be evaluated with conventional odd ratios methodology, which are routine in diagnostic evaluations and have been amply used in previous veterinary obstetric studies. Appropriate measures include estimates of sensitivity and specificity pairs, likelihood ratio of positive and negative result pairs, and Receiver Operating Characteristic (ROC) analysis along with confidence intervals.[53] However, in the past decade, analysis using ROC curves for clinical diagnosis has gained in popularity due to the advantages it has over more conventional statistical methodology.[43, 54] In fact, ROC analysis is considered a more robust analytical method in that it overcomes some of the limitations of traditional statistical evaluation.[27, 52, 55]

The overall accuracy of ROC curves, using UI as a screening test for the diagnosis of the predicted fetus number, can be compared visually at a glance evaluating the area under each curve (AUC). The curve closest to the uppermost left corner will have the greatest AUC[43, 55]. Therefore, it depicts curves that are best at discriminating between given states.[52, 54] This means, discriminating between goats with a given number of fetuses.

The calculated ROC curves can also be easily quantified using computerized algorithm's that calculate the AUC which lend themselves for determining statistical difference significance between calculated curves (e.g., Figure 2). Although orthodox

methods used to evaluate a diagnostic method or technology employ a calculated value for sensitivity and specificity, that is, they evaluate the proportion of goats correctly classified as having or not having a condition of interest. Authors argue that reporting only a single value for each UI metric is an over simplification of the real nature of the relationship between sensitivity and specificity since a diagnostic procedure has many values.[55] As a consequence, the criterion used to evaluate sensitivity and specificity varies and the UI metrics themselves are dynamic depending on the criterion threshold chosen.

A ROC plot was used to determine the relationship between the true positive probability (sensitivity) vs. the false positive rate probability (1-specificity) of pregnancy diagnosis results. The area under the probability curve (AUC) was also determined to test for statistical significance between results of fetus number diagnosis. As expected, when the true positive rate (pregnant goats diagnosed by UI as pregnant) increased, the number of goats determined to be pregnant, when they are in fact open, also increased.

As stated, on the ROC curve the highest criterion C is sought. That is, the higher the probability curve is from the diagonal, the better the fit and the more valid the UI diagnosis in terms of its specificity. The ROC curve for the number of fetuses diagnosed by means of UI at ±45 dPB was calculated. The sorting efficiency of the model provided individual curves as well as the corresponding AUC. As shown in Figure 2, all curves were above the reference curve of no-efficiency sloping at the 45º diagonal ($P<0.001$). This means that in terms of overall prediction ability, a UI scan interpretation that the goat is not pregnant has a sensitivity of 95%, followed by the ability to determine 3 fetuses at 90%, followed by the ability to diagnose the presence of twins at 88% and

finally, single fetuses were detected with a sensitivity of 78%. We have no biological and/or technical/equipment explanation to speculate for the reason why single fetuses show less sensitivity to UI scanning than observations where there are twins yet no different than observations where there are three fetuses.

Discussion

The use of ultrasonography has gained wide acceptance for consistent and early reproduction management evaluation[56-59] and for the application of a variety of assisted reproductive technologies.[60-63] Specifically in small ruminant veterinary practice UI has become the most efficient diagnostic tool.[7] UI-based pregnancy diagnosis has been compared to other diagnostic procedures such as progesterone, and pregnancy-associated glycoprotein assays and the results were deemed to be very accurate.[64]

In general, previous work with UI technology for pregnancy diagnosis and/or fetus number determination using goats has not included validation of the technique for accuracy, specificity and sensitivity. Validation to establish reliability under conditions of use is critical.[24, 65, 66] An additional hurdle for comparative discussion with other published results is that, with few exceptions, (for example[20, 64]) other documented results do not come from research designed to evaluate UI *per se,* but instead represent research where UI metrics were obtained to measure the effect of other independent variables pursuant to other objectives; pregnancy or litter size were not the primary response variables.

In addition to the lack or insufficient systematic evaluation of reliability, studies with small ruminants have not considered effects of production phenotype or parity on UI results. In this study we determined that both production phenotype and parity influenced UI metrics.

Pregnancy rate

Overall pregnancy rate determined at ±45 dPB and at kidding was 55% and judged to be consistent with other published results.[67-71] Both lower[72-75] and higher pregnancy rates[76-

[87] have been reported and other work has reported both higher and lower pregnancy rates in the same research setting.[88-98]

A 55% pregnancy rate could be generally considered low compared to natural service and non-restricted access of the buck to estrus presenting females as found in some studies: (82-91%),[99] (80-96%),[100] (95%),[101] (71-87%),[102] (61-77%)[103] and, using our own Alpine herd in past years, we have obtained 64 to79% pregnancy rates for 1st-time, hand-bred young and mature goats.[104] In this study, goats bred by natural means had a 1st-time service resulting in a 64% pregnancy rate The lower overall pregnancy rate, compared to that obtained by natural service, is attributed to: using unselected mature and young goats, use of hormonal estrus synchronization, and the breeding protocol described. In addition, we cannot rule out the possibility of refractoriness to gonadotropin effects (i.e., no ovulation) in the hormonal protocol[105,106] particularly in older multiparous goats who may have been hormonally synchronized in previous years.

Correlation between UI pregnancy status and kidding

The actual correlation value between pregnancy diagnosis at ±45 dPB and does kidding was 91% ($P<0.0001$). Although highly significant, both r and ρ values in this study were not as high as expected (i.e.,> 95%) since both are measuring the same response variable. Those results were corroborated quantitatively mainly by the low negative predictive value of 93.3% and somewhat low positive predictive value (precision) of 95.5%. An explanation for why UI detection had a high discrepancy has to do with variability introduced by the interface of the type of goat and the UI procedure itself, chiefly by behavioral differences between breeds and possibly due to the presence of a more dense

fiber coat in the Angora and Spanish breeds which, unless great care is taken at clipping, prevents appropriate contact between the abdominal wall and the UI scanning probe.

Correlation between fetus number determination by UI and litter size at birth

The Spearman rank correlation for the comparison of fetus number prediction and actual litter size was low at 84%. Three potential reasons are recognized for the discrepancy between the clinical diagnosis and actual results. These are: prenatal loss, increased difficulty while performing the scan due to a behavioral response when evaluating breeds not habituated to being handled (i.e., non-tractable goats), and difficulty in differentiating triplet and quadruplet fetuses because of image interference caused by increased uterine crowding.

Effect of goat production phenotypes and parities on UI validation metrics

Sensitivity of UI scanning used to establish pregnancy status or to determine fetus number was not influenced by production phenotype or parity. Overall sensitivity for pregnancy status was 95 ± 0.8 within production phenotype and parity. Overall sensitivity for fetus number was 93% ±0.8 for both production phenotype and parity with an average accuracy of 76 ±3.5. Although the general accuracy value obtained in this study is not directly comparable with previous research, Dawson and colleagues[21] found that the accuracy for determining singles, twins, and triplets at 7 wk of gestation was 82, 89 and 100%, respectively.

Specificity was influenced by production phenotype but not by parity. Dairy and fiber production phenotypes had specificities of 100 and 87.5, accordingly ($P<0.063$) whereas all parity specificities were in the 90's. It is possible that fiber goats, despite clipping

prior to UI scanning, due to their thick fiber coat (cashmere and mohair) still elicit interference preventing good contact between the animal skin and the UI scanning probe. Breed specific subcutaneous fat deposition or that found associated with abdominal omentum may also be a source of variation and would need to be confirmed independently, nevertheless it is known that assessment of fat depots is costly, time-consuming and involves a trained technician.[107]

Dawson and co-workers[20] obtained a specificity value of 100% for females evaluated at 35 and 49 dPB, reflecting that all does diagnosed as being open did not kid. In the present study overall specificity was 95%, however, specificity attained in this study with a greater number (n=173) of adult and young Alpine dairy production phenotype was also 100%.

Precision analysis showed that results of UI scanning were influenced by production phenotype and not by parity. Again, the presence of remnants of clipped fleece and/or cashmere in fiber goats may have influenced the levels of precision. Precision was significantly greater for dairy (100%) compared with the fiber production phenotype (91.7%). Parity was non-influential of precision.

Neither production phenotype nor parity influenced UI accuracy in this study. In comparison, Dawson and colleagues[20] found that with Alpine does and doelings UI accuracy for open females at 49 dPB was 100%**.** Accuracy for singletons at 35 d and at 49 d was 44 and 82%, respectively.

As observed in Tables 10 through 12, false positive rate (FPR), which also measures classical statistical inferential type I error or the probability of accepting a hypothesis as

true (*ie*., a pregnant diagnosis) when actually false (*i.e.,* an open goat) was unacceptably high to be of practical value for fetus number determination. That is, on average there was a 31.9 ±10.4 FPR for production phenotype and 35.1 ±4.9 for parity group.

Conclusion

Recapitulating, the purpose of this study was to describe the influence, if any, of goat production phenotype and parity when reproductive status and number of embryos is evaluated using B-mode UI technology and to include ROC statistical analysis in the assessment.

In summary, the use of transabdominal UI at 45 dPB to determine if a goat was pregnant or not pregnant for the most common production phenotypes and parity categories was shown to be consistent with the results obtained at parturition. To the contrary, using the same approach to estimate the number of embryos in utero, transabdominal UI technology was influenced by both production phenotype and parity and was deemed unreliable. Therefore, reliability of UI data generated for fetus number evaluation for dairy and meat production phenotypes as well as for multiparous and primiparous goats requires further evaluation due to the high variability and low clinical performance. Possible strategies to mitigate the problem would include any or a combination of the following: delaying observation from 45 days to 60 days, performing a second UI scan, perform a transrectal UI to determine the number of CLs present, use of additional recorded information such as breeding dates or progesterone analysis levels, for all parity groups except nulliparous goats previous records of litter size may be helpful and consideration must be given to perform rectal UI of the uterus.

In the context of the statistical evaluation for significant differences between the UI metrics generated for each treatment group, whether the evaluation is performed by traditional means (odds ratios and chi-square likelihood probability distribution) or by a more graphically oriented analysis with ROC analysis and statistical comparison of the

areas under the curve, the results yield to the same conclusions. However, the use of ROC analysis makes interpreting the results more efficient as it visually portrays in one image the location of the accuracy point threshold in relation to the remainder of possible threshold values. That is, it is useful for organizing values generated in a confusion matrix allowing for visualizing the classifiers and their performance expediently.

The value of ROC analysis is apparent when several alternative responses are possible such as different number of embryos detected. Using traditional analysis methodology it would be cumbersome to arrive at the best threshold and difficult, if not impossible, to compare different embryo number categories for inferential statistics hypothesis testing.

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Table 1. Summary of reproductive status as determined by ±45 dPB UI and scanning evaluation metrics (%).

	Ultrasound imaging		Parturition	
	40-45 d post breeding		±150 d post breeding	
Reproductive status	**Nº**	**%**	**Nº**	**%**
Pregnant	246	55%	247	55%
Non-pregnant	202	45%	201	45%
	448	100%	448	100%

	Sensitivity	Specificity	Precision	Accuracy	False positive rate	Negative predictive value
Avg=	95.2	94.5	95.5	94.8	5.5	93.3
±SE=	0.8	1.7	1.4	1.1	1.7	1.3

Table 2. Summary of fetus number as determined by ±45 dPB UI and scanning evaluation metrics (%).

	Ultrasound imaging		Litter size at term	
	40-45 d post breeding		±150 d post breeding	
Nº of fetus:	**Nº**	**%**	**Nº**	**%**
0	201	45%	201	45%
1	82	18%	69	15%
2	149	33%	132	29%
3-4-5	16	4%	46	10%
	448	100%	448	100%

	Sensitivity	Specificity	Precision	Accuracy	False positive rate	Negative predictive value
Avg=	92.8	66.5	62.2	75.8	33.5	93.3
±SE=	0.8	5.2	6.4	3.5	5.2	1.3

Table 3. Contingency table of most likely[a] pregnant by goats actually kidding.

	Count Total % Col % Row %	Most likely kidding (based on ±45d PB UI)		
		Open	Pregnant	
Actual goats kidding	Did not kid	191 42.63 95.02 94.55	11 2.46 4.45 5.45	202 45.09
	Kidded	10 2.23 4.98 4.07	236 52.68 95.55 95.93	246 54.91
		201 44.87	247 55.13	448

[a]**Most Likely:** Based on Nom. Logis. Reg. model: Most likely pregnant = Goats kidding.

Table 4. Contingency table of most likely[a] ultrasound imaging (UI-adj[b]) fetus number by litter size.

	Count Total % Col % Row %	Most likely UI-Adj fetus number				Count Total %	Total errors	RER (%)
		0	1	2	3			
Actual litter size at term	0	190 43.08 94.53 94.53	5 1.13 7.25 2.49	5 1.13 3.79 2.49	1 0.23 2.56 0.50	201 45.58	11	5.47
	1	9 2.04 4.48 10.71	35 7.94 50.72 41.67	28 6.35 21.21 33.33	12 2.72 30.77 14.29	84 19.05	49	58.33
	2	2 0.45 1.00 1.40	29 6.58 42.03 20.28	94 21.32 71.21 65.73	18 4.08 46.15 12.59	143 32.43	49	34.27
	3	0 0.00 0.00 0.00	0 0.00 0.00 0.00	5 1.13 3.79 38.46	8 1.81 20.51 61.54	13 2.95	5	38.46
		201 45.58	69 15.65	132 29.93	39 8.84	441	114	25.85

[a]**Most Likely:** Based on Nom. Logis. Reg. model: Most likely number of fetus=Litter size
[b]**UI-adj:** Litter size of 4 and 5 fetuses were not included (n=7).

Table 5. Criterion (*) values and coordinates of the ROC curve.

Criterion	Sensitivity	95% CI	Specificity	95% CI
≥ 0	100.0	98.5 - 100.0	0.0	0.0 - 1.8
>0 *	95.5	92.2 - 97.8	95.0	91.0 - 97.6
>1	0.0	0.0 - 1.5	100.0	98.2 - 100.0

Table 6. Likelihood ratios (LR) and predictive values (PV) of the ROC curve for pregnancy diagnosis by UI at ±45 dPB.

Criterion	+LR	95% CI	- LR	95% CI	+PV	95% CI	- PV	95% CI
≥ 0	1.0				55.1	50.4 – 59.8		
>0 *	19.2	18.4 – 20.0	0.1	0.02 - 0.10	95.9	92.7 – 98.0	94.6	90.5 - 97.3
>1			1.0				44.9	40.2– 49.6

Table 7. Overall ultrasound diagnostic metrics for pregnancy status.

	Sensitivity	Specificity	Precision	ACC	FPR	NPV
Average=	95.2	94.5	95.5	94.8	5.5	93.3
±SE=	0.8	1.7	1.4	1.1	1.7	1.3

Table 8. Ultrasound diagnostic metrics for pregnancy status by goat phenotype.

Phenotype	Sensitivity	Specificity	Precision	Accuracy	FPR	NPV
Dairy	96.7	100.0	100.0	97.7	0.0	93.1
Fiber	91.7	87.5	91.7	90.0	12.5	87.5
Meat	95.4	94.6	94.9	95.0	5.4	95.2
Average	94.6	94.0	95.5	94.2	6.0	91.9
± *Stand. Err.*	1.5	3.6	2.4	2.3	3.6	2.3

Table 9. Ultrasound diagnostic metrics: pregnancy status according to goat parity.

Parity	Sensitivity	Specificity	Precision	ACC	FPR	NPV
Multiparous	95.8	96.4	97.2	96.1	3.6	94.6
Nulliparous	96,6	92.6	91.8	94.4	7.4	96.9
Primiparous	94.9	96.2	97.4	95.4	3.8	92.6
Average	95.8	95.1	95.5	95.3	4.9	94.7
± *Stand. Err.*	0.5	1.2	1.8	0.5	1.2	1.3

Table 10. Overall ultrasound diagnostic metrics for fetus number determination.

	Sensitivity	Specificity	Precision	ACC	FPR	NPV
Average=	92.8	66.5	62.2	75.8	33.5	93.3
±SE=	0.8	5.2	6.4	3.5	5.2	1.3

Table11. Ultrasound diagnostic metrics for evaluation of fetus number according to goat production phenotype.

Phenotype	Sensitivity	Specificity	Precision	ACC	FPR	NPV
Dairy	94.3	51.9	56.9	69.0	48.1	93.1
Fiber	91.7	87.5	91.7	90.0	12.5	87.5
Meat	92.1	65.0	52.5	73.0	35.0	95.1
Average	92.7	68.1	67.0	77.3	31.9	91.9
± *Stand. Err.*	0.8	10.4	12.4	6.4	10.4	2.3

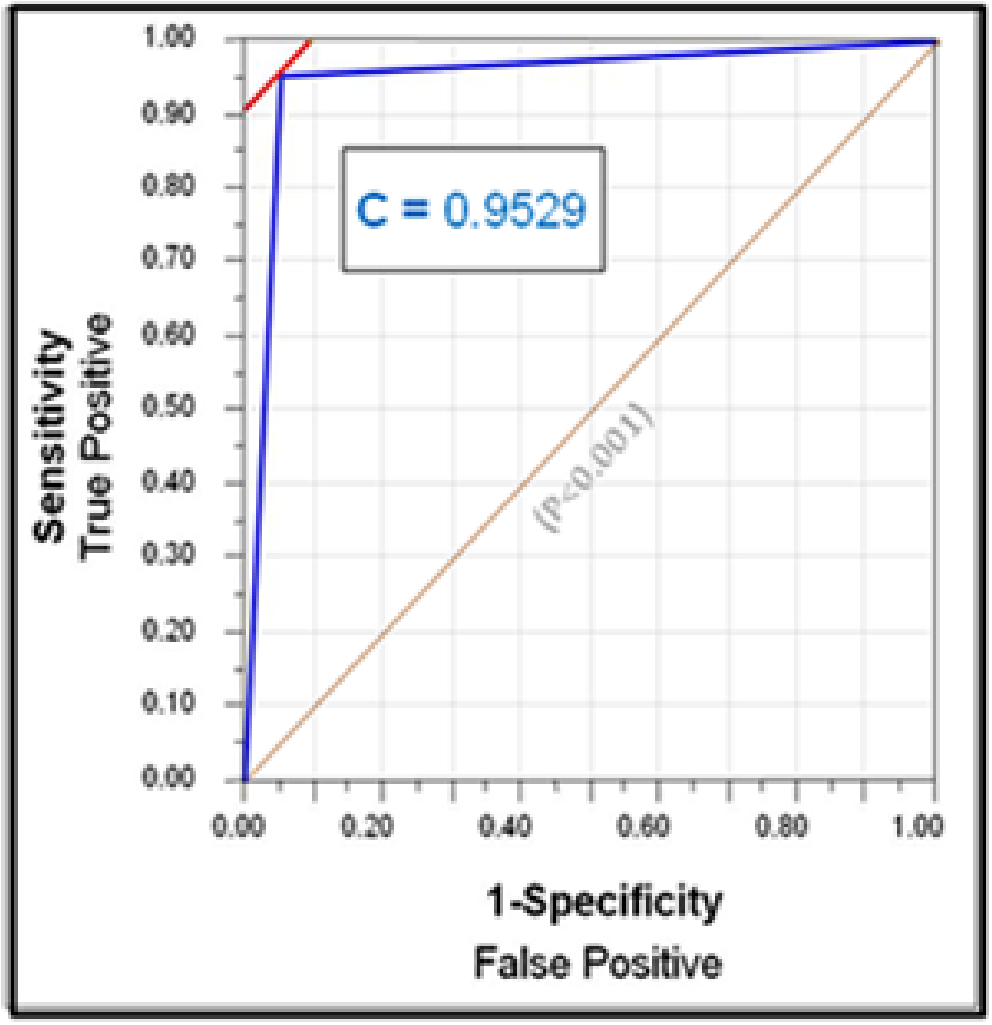
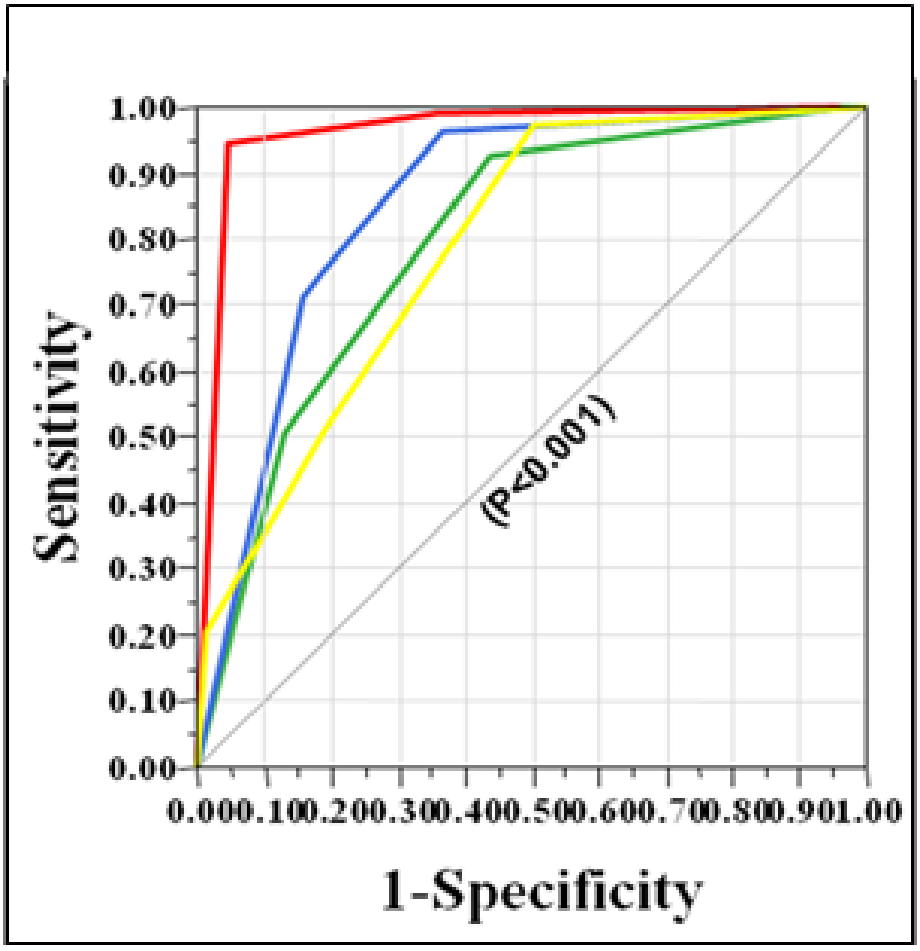

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Sensitivity
True Positive
C = 0.9529
(P<0.0001)
0.00
0.20
0.40
0.60
0.80
1.00
1-Specificity
False Positive

Figure 1. Receiver operating characteristic (ROC) for goat pregnancy diagnosis by ultrasonography at ±45d post breeding. The 45° diagonal represents the line of no influence. C (95.3%) is the area under the curve as a coefficient of 1. The ROC graph depicts the true-positive proportion plotted against the false-positive proportion for alternate settings of a decision criterion. The idealized curve would pass through the upper left corner.



Legend	N° of fetuses	C
	0	0.9628
	1	0.7960
	2	0.8528
	3	0.7879

Figure 2. Receiver operating characteristic (ROC) for goat fetus number detection by ultrasonography at ±45d post breeding. The plot illustrates the relationship between the true positive probability (sensitivity) vs. the false positive rate probability (1-specificity) of pregnancy diagnosis results. The area under the probability curve (AUC) is used to test for statistical significance between results of fetus number diagnosis. In the graph when the true positive rate (pregnant goats diagnosed by UI as pregnant) increases, the number of goats determined to be pregnant, when they are in fact open, also increases. The highest criterion C is sought. The higher the probability curve is from the diagonal, the better the fit and the more valid the UI diagnosis in terms of its specificity. As shown, all curves lay above the reference curve of no-efficiency which slopes at the 45º diagonal.

Appendix A. Confusion matrix and calculation of ultrasound imaging diagnostic validation metrics.

		Pregnancy status or embryo Nº (as confirmed by kidding or litter size)		
		Positive	***Negative***	
Test outcome	***Positive***	**True Positive (TP)**	**False Positive (FP)** (Type I error, P-value)	→ **Positive predictive value (PPV or precision)**
	Negative	**False Negative (FN)** (Type II error)	**True Negative (TN)**	→ **Negative predictive value (NPV)**
		↓ **Sensitivity**	↓ **Specificity**	↘ **Accuracy**

Sensitivity = TP / (TP + FN)

Specificity = TN / (TP + FN)

Precision = TP / (TP + FP)

NPV = TN/ (FN + TN)

Accuracy = (TP + TN) / (TP + FP + FN + TN)

CHAPTER IV

REPRODUCTIVE PERFORMANCE IN GOATS FOLLOWING SYNCHRONIZATION OF ESTRUS AND OVULATION USING FIXED-TIME ARTIFICIAL INSEMINATION OR NATURAL SERVICE

Abstract

Reproductive performance (RP) of unselected dairy, meat, fiber, and meat×fiber crossbreds goat phenotypes (n=879 breedings) was evaluated using ultrasonography at an average of 46 ± 4 days post breeding. All goats ranged from 1.5 to 11 years of age with an average herd age of 4.1±1.6 y. The effect of progesterone (P4) time exposure, chorionic gonadotropins, and type of breeding procedure on RP was evaluated for first-time bred goats (n=533). Data were fitted to polytomous logistic regression models using goat breed, breeding/kidding season, age, parity, sire, artificial insemination (AI) technician and breeding number as blocking variables. RP was evaluated through reproductive efficiency traits (RET): conception rate (CR), pregnancy rate (PR), fertility (F), prolificacy (Pr), and fecundity (Fc). RET for synchronized, fixed-time bred goats were: CR=57%, PR=50%, F=61%, Pr=1.8, Fc=1.09, and KR=61%, compared to goats

naturally serviced with no synchronization: CR=79% ($P<0.001$), PR=67% ($P<0.001$), F=53% ($P<0.02$), Pr=1.7 ($P<0.008$), Fc=0.89% ($P<0.0001$), and KR=52% ($P=0.07$). Fixed-time natural service resulted in the highest PR with 66% compared with 46% PR for fixed-timed intra-uterine AI ($P<0.0001$) or 27% PR attained with fixed-time trans-cervical AI ($P<0.02$).

In conclusion, synchronization protocols with 5-6 d use of P4 and a combination of eCG/hCG reduced reproductive efficiency. The use of eCG/hCG in P4-based synchronization protocols affected CR of non-treated goats compared to goats receiving 1.75 mL PG600 ($P<0.02$). As expected, breeding procedure had a significant effect on all 6 RETs (odds ratios) studied: ($P<0.044$) for CR, ($P<0.025$) for Pr and ($P<0.001$) for the remaining RETs. Fixed-time breeding in naturally serviced goats decreased C and PR ($P<0.007$) but not Kr ($P>0.071$) and F, Pr, Fc ($P>0.410$).

Introduction

In commercial goat operations, increasingly estrus/ovulation synchronization (E/OS) induction is done by administering progestagens to extend the luteal phase of the estrous cycle.[1, 2] Commonly the delivery is in the form of intravaginal implants which are controlled internal drug release (CIDR) dispensers based on an inert silicone elastomer commonly impregnated with progesterone,[3-7]

Progesterone (P4) or its analogues, have been traditionally administered for a period similar to the species-specific duration of the corpus luteum (CL) spurium, and are used to decrease production of GnRH by the hypothalamus.[8] P4 also down-regulates estrogen nuclear receptors in cells of the uterus[9, 10] This P4 negative feedback inhibition precludes behavioral estrus and postpones ovulation by preventing luteinizing hormone (LH) surges in the follicular and late follicular stages of the estrous cycle[11, 12] until P4 influence is removed.[13, 14]

Contemporary research considering the appropriate time of P4 exposure has used the information available on the wave nature of follicular development, instead of using the length of the luteal phase in goats.[15, 16] This approach has resulted in E/OS protocols that expose the animal to P4 for shorter periods of time, rather than the traditional 12 to 14[7] or 9 to 16 days[1, 17] or 16 to18 days[18, 19] or even 18 to 21 days[20] exposure time. Field trials have validated the use of P4 exposure to only 5 to 6 days while providing a luteolytic dose of prostaglandin at time P4 is administered and the use of equine chorionic gonadotrophin (eCG) or estradiol benzoate (EB) at time of P4 removal.[21-23]

Menchaca et al (2007)[24] have shown that the 5 to 6 d short-term protocol induced similar concentrations of P4 among treated goats and in combination with eCG or EB results in similar increase in estradiol-17ß and a comparable LH surge capable of eliciting ovulation in 86.7% of treated females at a consistent, 60h interval, after the end of P4 exposure. Conversely, prolonged P4 has been shown to lead to reduced fertility in ewes,[25] increased embryonic mortality, gamete transport hindrance in the female reproductive tract, insufficient follicular maturation, and delayed ovulation.[23, 26, 27]

Currently in the U.S. no reproductive hormone has been approved for goats since last reviewed.[28] This regulation limits the use of some pharmaceutically prescribed hormones to an "extra-label" use as specified under the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 as published by the Food and Drug Administration Compliance Policy Guide (FDA, 2009).[29] The gonadotropin-containing commercial product PG600®, designed for porcine reproduction, is the only available pharmaceutical which contains eCG that, when used in domestic farm species other than the horse, in doses ranging from 400 IU to 1000 IU,[30] characteristically elicits both LH and follicle stimulating hormone-like (FSH) activities.[31, 32] Because of this dual biological effect, eCG has been used to induce ovarian follicular growth, both for goat enhanced ovulation and for estrus induction and synchronized breeding programs.[33]

PG600 also contains the luteotrophic agent human chorionic gonadotropin (hCG), a peptide hormone produced in pregnancy by the syncytiotrophoblast layer of the primate placenta.[34, 35] In recent years hCG has been shown to have a wealth of functions in the placenta, uterus and possible in the fetus during pregnancy.[36] Nonetheless, the biological function of hCG germane to this study, is to prevent the disintegration of the ovarian CL

verum by interacting with the LH/CG receptor and, in this manner, promote sustained P4 secretion by ovarian corpus luteal cells.[36, 37] Although P4 inhibits hCGα gene transcription in human trophoblast cells,[38] importantly, in goats where the CL is the sole progestational hormone source that maintains pregnancy,[39] hCG not only behaves much like FSH and LH but it is not inhibited by a rising level of P4.

To our knowledge, no large scale goat reproductive performance study has been published regarding the Southwestern environment conditions of the U.S. Likewise the short-term (5-6 d) P4 priming protocol with eCG/hCG combination as a substitute for E/OS protocols using eCG alone, has not been addressed. Furthermore, our previous unpublished transcervical artificial insemination work led us to believe that although a short P4 interval was successful in bringing a large proportion of females in estrus, the procedure diminished reproductive efficiency by making insemination less successful by the interplay of a set of influential factors poorly characterized so far. We postulate that short P4 priming in combination with PG600 and fixed-time breeding reduces the efficiency of economically relevant reproductive traits.

In summary, the objectives of this study were to evaluate breeding records in a large goat herd to characterize reproductive performance, compare those results with the performance generated using estrus/ovulation synchronization protocols based on variable-term progesterone exposure combined with use of eCG and hCG, and breeding by fixed-time natural service, transcervical and intrauterine artificial insemination.

Materials and Methods

Animals

This study was conducted under field and research facility conditions using the guidelines of the Animal Care and Use Committee at the American Institute for Goat Research (AIGR), Langston Oklahoma (*Lat*. 35.945° N *Long.* -97.255° W, 292 m.a.s.l.) during the reproductive seasons (September through January) of 2006 through 2008 and their respective kidding seasons. Daylight hours ranged from 12.8 h to 9.6 h from the beginning to the end of the breeding season.

The study utilized unselected mature and young goats ranging in age from 1.4 to 11 years of age with an overall average age and standard deviation of 4.1 ±1.5 yr.

Animal Management

The Alpine herd consisted of non-lactating goats managed semi-extensively on Bermuda or Sudan grass as well as being placed in wheat pastures when fresh forage was available. Nutritional supplementation was given when necessary using a dry-goat ration (ME 2.3 Mcal/kg and TP 14.5%). Bucks had ad-libitum access to local prairie mixed grasses, Bermuda grass or Sudan grass hay as well as wheat hay. Additionally, bucks were supplemented with a maintenance ration (ME 2.2 Mcal/kg and TP 12.6%) according to body condition.

The meat and fiber goat herd composition was also that of dry animals during the breeding season with exposure to the natural decreasing photoperiod characteristic of the fall season. All goats were managed extensively on native Oklahoma mixed grasses and wheat pasture when fresh forage was available. As needed goats were supplemented with

either a low or high protein commercial custom-manufactured goat pellet supplement (Stillwater Milling Co. Stillwater OK) with13.3 and 20.3% CP, respectively. Hormone dosing, artificially inseminating and ultra sound scanning was done in indoor facilities. All goats were provided fresh water and had free access to mineral supplement licking blocks. All goats were under veterinary care and were treated regularly for internal parasites with anthelmintics (i.e., Cydectin®, Fort Dodge, Animal Health, Fort Dodge, IW, or Valbazen®, Pfizer, Animal Health. Exton PA, or Levazole®, Schering-Plough Animal health Co., Summit, NJ), all had access to portable plastic or metal shelters. Goats were cared for and monitored daily by farm personnel.

Evaluation of pregnancy diagnosis by ultrasound imaging

Ultrasound imaging (UI) for pregnancy diagnosis and to determine number of fetuses was evaluated for accuracy, specificity and sensitivity. Details of the technical evaluation included statistical correlation analysis, percent error rate, symmetry of agreement (statistic κ) and operating receiving curves will be bee published elsewhere (presently the information is provided in this dissertation in Chapter III).

Study variables

Three primary independent variables (treatments) were considered: 1) Progesterone delivery time (*none* (N); *short* (S): 5 d; *long* (L): 10-14 d and *extra-long* (L): 24 d), 2) Type of fixed-time breeding: Trans-cervical artificial insemination (TrAI), laparoscopy-aided intrauterine insemination (LAI) and, natural service with penned group females (NSp) for both, non-synchronized fixed-time breeding, and non-synchronized, non-fixed timed breeding, and 3) Dose level of eCG/hCG (PG600): None, 140/70 units (1.75 mL) or 400/200 units (5 mL), respectively. Secondary independent variables (blocking

covariates) evaluated were: Goat breed as described previously: A, Ang, B, S, SL, and XB, TrAI technician (2), female age as a continuous or categorical ordinal variable (**1:** ≤3 y; **2:** >3 and ≤4; **3:** >4 and ≤5; **4:**>5, parity (nulliparous, primiparous and multiparous) and breeding/kidding season; 2006-7, 2007-8 and 2008-9 respectively.

Reproductive performance was evaluated using reproductive efficiency traits (RET): conception rate, pregnancy rate, fertility, prolificacy, fecundity and kidding rate. The calculations used to quantify each response variable outcome were as follows:

Conception rate (CR)

Conception rate was defined and calculated in two ways: as the number of conceptions (i.e., attached embryos) per bred female CR_{embryo} or, alternatively, in terms of the number of females conceiving per group of bred females $CR_{maternal}$. In both CR formulas below, the number of females bred includes does and doelings with a documented breeding.

$$\boldsymbol{CR_{embryo}} = \frac{\text{N° of attached embryos}}{\text{N° of bred females}} \times 100$$

$$\boldsymbol{CR_{maternal}} = \frac{\text{N° of does with at least 1 embryo}}{\text{N° of bred females}} \times 100$$

As depicted in Figure 1 it was considered that conception (i.e., embryo attachment) had occurred if at least one embryo was presumed to have attached when there was no return to estrus by day 17 up to day 25 PB or if there was a return to estrus, after having been bred, in an atypical estrous cycle period of days (*i.e.,* > 24 to <39 for first cycle post breeding and > 24 to <39, > 45 to < 60 d and >66 < 81 for second cycle post breeding).

Pregnancy rate (PR)

Pregnancy rate is defined as the number (N°) of pregnant females in relation to the number of females bred. A pregnant female describes a goat with at least one UI confirmed fetus (a positive viable pregnancy was recorded when a fetus, cotyledons and/or fetal vesicle fluids were observed) at a target time of 45 dPB. Number of females bred includes does and doelings with a recorded breeding.

$$\boldsymbol{PR} = \frac{\text{N° of females UI diagnosed pregnant}}{\text{N° of females bred}} \times 100$$

Kidding Rate (KR)

Kidding rate can be defined in various ways: as the number of kidding females as a function of the number of females: exposed to bucks or bred or conceiving or fertile or diagnosed pregnant. The number of kidding females represents goats with at least one kid delivered dead or alive at term. Number of females conceiving represents females diagnosed with at least one fetus.

$$\boldsymbol{KR} = \frac{\text{N° of kidding females}}{\text{N° of females conceiving}} \times 100$$

Fertility (F)

Fertility includes the number of females that gave birth (does and doelings), goats that experienced prenatal losses, and does whose offspring died at peripartum (*e.g.,* dystocia, stillbirth, and physical gross anomalies). Breeding group refers to all females exposed to a buck but not necessarily bred does (i.e., females that did not exhibit estrus, and therefore not mated).

$$\boldsymbol{F} = \frac{\text{N° of fertile females}}{\text{N° of females in breeding group}} \times 100$$

Prolificacy (Pr)

Prolificacy was given by the number of kids born alive divided by the number of females kidding. Number of kids born alive excludes: abortions, stillbirths, pregnancies terminated and kids dead as a result of dystocia. Number of kidding females represents females with at least one kid delivered at term dead or alive.

$$\boldsymbol{Pr} = \frac{\text{N° of kids born alive}}{\text{N° of females kidded}} \times 100$$

Fecundity (Fc)

Number of kids born alive as above and number of females mated in the breeding group includes all females placed in a breeding treatment that were inseminated or naturally serviced, hence, it excludes goats not coming in estrus in the NNT group (goats in other breeding groups were timed inseminated whether or not estrus signs were evidenced).

$$\boldsymbol{Fc} = \frac{\text{N° of kids born alive}}{\text{N° of females mated in breeding group}} \times 100$$

Estrus and ovulation synchronization (E/OS) protocol

A total of 879 goats were randomly assigned to one of three P4 exposure treatments (long, short or x-Long) (Figure 2). In addition to the aforementioned, two non-synchronized control groups were used. To assess the influence of fixed-time breeding on RP a control group of non-estrus synchronized goats (N) assigned to fixed-time natural service were bred on a non-synchronized, naturally occurring estrus 24 h after the onset of standing estrus as determined from breeding marks (see Figure 3). A second cohort of

control goats (NNT) was placed with bucks and allowed to breed one time when both male and female were receptive.

All goats assigned to estrus synchronization treatment groups (of variable length of P4 exposure time) received an intravaginally-placed silicone elastomer CIDR-G® containing 300 mg of P4 (Eazi-Breed CIDR, Pfizer. Rydalmere, New Zealand). CIDR's were monitored to ensure retention. In the event that a device came off it was replaced immediately. Comparison (control) groups did not receive any hormonal treatment.

A second experimental estrus synchronization group was included in the long P4 exposure group where P4 was embedded with appropriate tool as an ear insert. The source of hormone was one-half of a 6-mg implant of Syncro-Mate-B® (SMB); (Sanofi Animal Health Inc. Overland, KS) for a total of 3 mg of Norgestomet placed subcutaneously in the outer surface of the ear. After 12 d the insert was removed through a small skin incision made over the implant.

Hormone treatment protocol for estrus synchronization also included an IM dose of PG600® (Intervet Inc. Millsboro, DE) at 5 mL (400 IU of eCG and 200 IU of hCG) or 1.75 mL (140 IU of eCG and 70 IU of hCG) given 24 h prior to CIDR removal and 2 mL of Lutalyse® (Pfiser. New York, NY) containing dinoprost tromethamine equivalent to 5 mg dinoprost/mL given IM immediately after P4 removal (CIDR or SMB).

Fixed-time breeding

All goats assigned to an experimental estrus synchronization protocol and breeding treatment group were bred 48 to 50 h after removal of P4 using standard procedures (see detail below) for laparoscopy-aided intrauterine artificial insemination (LAI),

transcervical AI (TrAI) or by fix-timed natural service of penned goats (NSp). Breeding was attempted whether or not overt estrus signs were observed. All bred goats were placed 5 to 7 d before their next scheduled estrus with bucks fitted with a breeding marking harness.

To assess the influence of fixed-time breeding on RP, a control group of non-estrus synchronized goats (N) were monitored for standing estrus using epididymectomized bucks (teasers) fitted with marking breeding harness. Twenty four h after a female was reported in standing estrus or was observed to have been marked it was taken to the buck for breeding. Bucks were allowed to breed females in estrus only once and does were removed from the pen immediately after the first breeding (see Figure 3). A second cohort of control goats (NNT) was placed with bucks and allowed to breed when both male and female were receptive. In these latter cohort, Bucks were also allowed to breed females in estrus only once and does were removed from the pen immediately after the first breeding.

All frozen semen for this study was purchased from two commercial vendors (Reproductive Enterprises, Stillwater, OK or Bio-Genics Ltd. Salmon, ID). Thawed-frozen semen used originated from 37 different sires (Boer and Alpine breeds) and custom frozen semen collected in previous years from one Tennessee Stiff-Leg breed stud sire.

Transcervical artificial insemination (TrAI)

TrAI were performed by two trained technicians. Insemination was accomplished using straws containing 0.5 mL of semen at a concentration of 100 and 120 million sperm/mL (Reproductive Enterprises, Stillwater, OK or Bio-Genics Ltd. Salmon, ID, respectively). Non-TrAI or partial TrAI were identified by the number of cervical annular folds (rings) that the AI inseminating tip was able to overcome in its path to the uterine body, as judged by the AI technician. For either LAI or TrAI only one, randomly selected (within breed), semen straw was used per inseminated goat. Standard AI procedures were used[40] with minor modifications.[41]

Intrauterine laparoscopically-aided artificial insemination (LAI)

Intrauterine artificial insemination was carried out by laparoscopic means by one technician using thawed-frozen semen. The technique was slightly modified from other published protocols,[42-44] as follows:

Twenty four h before LAI female goats were weighed and the area anterior to the udder and posterior to the umbilicus was short-clipped. Goats were isolated in individual pens, food-fasted for 24 h and deprived of water for 12 h prior to insemination.

Goats were pharmacologically sedated with 0.15 to 0.20 mg/Kg of body weight IM dose of X-Ject SA®, Butler Animal Health Supply. Dublin, OH) containing 20 mg/ml Xylazine. Fifteen to 20 m later (or more time if necessary for full sedative effect to ensue) the animal was placed in a dorsal recumbent position on top of a matted laparoscopic mobile table with a tilting cradle. All four legs were restrained by strapping to the table leg holds. While in horizontal position the shaved area was scrubbed clean

with surgical scrub Nolvasan®, Fort Dodge Animal Health. Fort Dodge, IA and disinfected with 70% isopropyl alcohol. Avoiding visible blood vessels two points for trocar entry were selected an marked at about 2 cm cranial to the area of anterior udder attachment and 1 and 5 cm left of the mid-ventral line. At the marked point of trocar entry, both areas were locally anesthetized by infiltrating the subcutis with an injection of 0.5 mL lidocaine (2% lidocaine hydrochloride; Butler Animal Health Supply. Dublin, OH). Stab incisions through the abdominal wall were made with a #10 scalpel surgical blade at the pre-marked, lidocaine injected points of entry. With the head down the cradle was tilted 30º to 45º with respect to the horizontal and locked in position. Prior to and in between intervened animals all laparoscopic instruments were kept submerged in Nolvasan® solution (Fort Dodge Animal Health. Fort Dodge, IA). Before using laparoscopic instrumentation each piece was fully irrigated with physiologic saline (0.9% NaCl).

A10 mm × 10 cm trocar and cannula set was inserted through the abdominal wall pointed slightly caudal, the abdomen was insufflated with filtered atmospheric air or CO_2 directly through the cannula equipped with a two-way stopcock. The second trocar and cannula was inserted, the trocar removed and replaced by an 18 cm Ilumina Panview endoscope (25^o). Endoscopic lighting provided through a fiber optic cable was attached and powered by either an Olympus CLK-3 with built-in air pump (Olympus Optical Co. Ltd. Japan) or a Wolf 5000.40 transilluminator (Cine Arc. R. Wolf medical Instruments Corp. Rosemenont, IL) used in combination with bottled regulated delivery of CO_2. When necessary the omentum folds were instrumentally pushed away from the reproductive tract (towards the diaphragm) to permit direct visibility of uterine cornua. A second

operator prepared the inseminating gun which featured a 20Ga × 9.5 mm laparoscopic needle at the end a 30.5 cm long sheath (Reproduction Resources. Walworth, WI).

The same frozen semen thawing procedures were followed for LAI as those used and described previously for TrAI (Loetz, 2006). Thawed-frozen semen was loaded in to the barrel of the inseminating gun and using pre-established calibration marks placed in the insemination metal rod plunger, which pushes semen out of the semen straws, approximately half of the semen straw volume (*i.e.,* 0.125 ml) was placed into the uterine lumen of each of the two horns at the uterine greater curvature approximately 2 to 3 cm cranial to the outward uterine body bifurcation by penetrating each uterine wall with a 15 mm needle placed at the tip of the LAI plastic sheath.

At the end of the laparoscopic insemination, remaining abdominally insufflated air or CO_2 was allowed to vent out and instruments removed. Abdominal punctures were sprayed with an anesthetic/antimicrobial topical solution Dermacool®. Virbac Animal health. Fort Worth, TX). Each abdominal opening was stapled close with a skin stapler Reflex®, ConMed Corp. Utica, NY.

When necessary, reversal of the anesthetized condition caused by xylazine was accomplished using an intramuscular dose of 2 mg/kg of body weight antagonist Tolazine® (tolazoline HCl, 100 mg/mL) (Lloyd Labs. Shenandoah, IW). Goats were supervised until fully recovered and post-operative procedures followed as instructed by herd veterinarian. Skin staples were removed one week after LAI procedure.

Natural service (NS)

Three weeks prior to each breeding portion of the study all bucks were evaluated by standard breeding soundness exam (BSE) with semen obtained by electro-ejaculation. Only 7 of 12 adult bucks that passed the BSE (on the first breeding season and the same 7 bucks confirmed sound on the 2nd breeding season) were used throughout the study.

Each buck was fitted with a color marking breeding harness and to minimize erroneous repeated breeding recordings a new, but differently colored crayon, was replaced as needed. No more than 15-20 breeding females were placed with each male at one time. All animals in the natural service breeding group were frequently checked for crayon markings and other characteristic signs of estrus throughout normal farm working hours (6:00 to 17:00). A doe was presumed to have been bred when her rump was colored-marked by the marking crayon and was immediately separated from the breeding group. Bucks were removed from their breeding group once all females had been marked or 24 h after exposure, whichever came first.

Does bred by NS where no synchronization was used and with no fixed-time breeding were placed with bucks irrespective of their time in the estrous cycle and kept with the males until bred. After a breeding was documented (rump color marked) the female stayed in the breeding pen for not more than 2 weeks. If the doe came back in estrus for a 2nd, 3rd or 4th time she was placed again with a breeding buck (see Figure 3).

Pregnancy diagnosis and fetus number evaluation

Reproductive status and fetus number evaluation was performed in accordance to established procedures.[45] In brief, pregnancy diagnosis was done at approximately 40 to

50 days post breeding (dPB) by mid-ventral external ultrasonography examination using a portable ALOKA SSD-500V (Aloka Co. Ltd., Japan) equipped with a 3.5 MHz linear array transducer (UST-934N) mounted on an external scanning device. All inconclusive results were repeated 2 to 3 weeks later. Results of pregnancy detection by UI were evaluated (confirmed to actual fertilization date) at the end of the study by backtracking 150 days from actual parturition day. Likewise the number of fetuses detected at time of UI was compared to the actual litter size obtained at full term.

Validation of ultrasound imaging procedure

Results of the validation for the ultrasound procedure are provided in Chapter III. Results include: a) accuracy of ultra-sound pregnancy detection at 47-days post breeding which was evaluated by comparing results against actual parturition data by Pearson's correlation and by calculating the Agreement Statistic (κ) for matching levels across two categorical variables, b) sensitivity and specificity of UI diagnosis for pregnancy and fetal number, and c) the relative error rate, likelihood ratios and predictive values.

Statistical Analysis

Sample size

Treatment effects were analyzed using data generated by 533 first-breeding goats. However, a total 1005 breeding records were accumulated and reviewed since goats open to a particular breeding were assigned to be re-bred a 2^{nd}, 3^{rd} or 4^{th} time by means of natural service. Of the 533 first-breeding goats, 416 (78%) observations were unique. Considering the entire database (n=1005) the percentage of unique observations was 42%. Remaining goats were evaluated more than once (*i.e.,* goat was rebred by NSp if open, or the same goat was used in a different breeding year).

Randomization

All first breeding goats (n= 533) were randomly assigned to a particular treatment (breeding procedure and synchronization protocol).

Treatment comparisons

Only the first breedings (n= 533; 61%) were used for statistical analysis of treatment effect comparisons for the various response variables. When a second or more services took place (n= 346; 39%) these occurred with no hormonal synchronization and/or with or without fixed-timed breeding and were reported and recorded in the breeding database to asses overall herd RP potential. Central tendencies were expressed as the arithmetic mean ± SD or ± SE, as appropriate. All mean differences were considered statistically significant if the *p*-value was less than 0.05 unless otherwise stated.

Statistical model. Reproductive efficiency variables containing binary states (*i.e.,* pregnant or not pregnant, kidded or not kidded) were analyzed using a logistic regression model:[46, 47]

$$\hat{P} = \frac{e^{b_0+b_1+bx_1}}{1+ e^{b_0+b_1+bx_1}}$$

Analysis of the difference between proportions, in terms of comparative outcome of several 2×2 tables for different classes of covariates of interest, adjusted for the magnitude of the proportions of occurrences being compared, were performed by means of odds ratios: [48, 49]

$$\boldsymbol{OR} = \frac{p_1/(1-p_1)}{p_2/(1-p_2)} = \frac{p_1/q_1}{p_2/q_2} = \frac{p_1 \times q_2}{p_2 \times q_1}$$

where,

an odds ratio (OR) of 1 indicates that X and Y are independent and that the probability of an event can be expressed in terms of their marginal probabilities. That is, the condition or event under study is equally likely to occur in both groups. An OR greater than 1 indicates that the condition or event is more likely to occur in the first group. And an OR less than 1 indicate that the condition or event is less likely to occur in the first group compared to the probability of occurring in the 2nd group. When needed conversion from an OR to probabilities was performed as follows:

$$\boldsymbol{OR} = \frac{\text{Probability}}{1 - \text{Probability}} \qquad \textit{and,} \qquad \boldsymbol{Probability} = \frac{\text{OR}}{1 + \text{OR}}$$

Statistical inference for odds ratios OR significance between P4 treatments, within breeding procedure, were analyzed by use of the Chi-square (χ^2) test with P values corresponding to two sided tests; with one degree of freedom (JMP, 2011).[50]

$$\boldsymbol{\chi^2} = \frac{\sum\left(\left(\left|\text{Observed frequency} - \text{Expected frequency}\right|\right) - 0.5\right)^2}{\text{Expected frequency}}$$

Data adjustments

Age group. For purposes of forcing alphabetical sorting in predicted profile graphics, age group originally coded 1 through 4 according to age, was recoded: A1 through D4 (and called "age cohort" to differentiate it from "age group")

Pregnancy. A total of 879 breedings were recorded, of these, 205 were excluded. Of these 205 exclusions, 142 were not within the target range of 45 ± 10 dPB and 63 were goats late in the reproductive season that went unreported or were not presented to be

scanned by error. The resulting group (n=674) was called “Preg-adj1”. A second adjustment was necessary in order to exclude 226 goats that were not scanned as they had been determined to have suffered some form of pregnancy loss. The resulting group (n=448) was called “Preg-adj2”.

Breeding year. As shown on figure 4, data included two breeding years (2007 and 2008) and a partial breeding season in 2006. The data from 2006 contributed 2.3% to the total observations and was judged to be insufficient (n=20) to validly use it as a categorizing variable. Therefore, all 2006 data was re-coded as “year 2007”.

Results

Goats of six breeds were represented: Alpine (A), Angora (Ang), Boer (B), Spanish (S), Tennessee Stiff Legs (SL) and various percentage Boer × Spanish crosses (*i.e.,* ½, ¾, and $^{5}/_{8}$); hereafter referred to as cross-breds (XB). All goats ranged from 1.5 to 11.0 years of age with a herd average and SD of 4.1 ± 1.6 y. As shown in Figure 5, at time of breeding the overall mean and ±SD of body weight was 57.9 ± 9.8 kg and the most common body condition score (BCS) on a scale of 1 to 5 was 2.75 according to breed.

Evaluations

A total of 879 breeding records were included for this study (Table 1). The appraisal incorporated pregnancy data and fetus number resulting from UI at 46 ± 4 dPB. Verification *a posteriori* of pregnancy status and fetus number was done with kidding rate and litter size by using actual parturition data (gestation length 149 ± 4 d).

Of the 879 total breeding group, 61% (n=533) of the goats correspond to the first breeding attempt. Experimental treatments were assigned exclusively to these goats. Hence, statistical comparisons for main treatment effects were evaluated using only the first-time breeding group. The remaining 39% (n= 346) were bred up to a 4th time, overwhelmingly (97%) by natural service on non-synchronized spontaneous estruses.

Seven goats left the herd in the course of the study (4 correspond to goats culled due to reproductive-unrelated health issues and three animals died before data could be collected) and 3 records were discarded (2 from a goat not bred two estrus cycles due to an injured leg under treatment and one where CIDR placement had injured the cervix).

The remaining 7 records were used since reproductive information (*i.e.,* breeding and UI pregnancy diagnosis) had been collected prior to their departure from the herd.

Breeding group - synchronization and expression of estrus

Of the total 879 goats in the breeding group, 352 (40%) goats were hormonally synchronized. However, these 352 goats represent 66% when considering solely the 533 goats that were bred for the first time.

Fourteen goats (1.5%) were artificially inseminated a second, some a third and few a fourth time (Table 1). Apart of the 14 artificially inseminated goats described, if a goat was serviced more than once (n= 346) these latter breedings occurred on a natural (spontaneous) estrus and without fixed-time breeding by exposure and mating to a buck; all serviced goats were reported and recorded in the breeding database to asses overall herd RP attained.

A total of 12 goats (1.4%) did not express estrus and were not bred. Of the 533 first-time-breeding goats assigned to a particular breeding treatment group, 8 goats (2.3%) of the synchronized group (n=352) did not respond to the E/OS protocol treatment. Thus, did not come in estrus. From these latter 8 goats, 7 goats were part of the extra-long P4 exposure synchronization protocol group and were not marked by a breeding buck.

Of the 533 first-time-bred breeding group 87 (17%) were not scanned or the scanning was not performed at the pre-established time which prevented its use in some measures of RETs analysis (*i.e.,* pregnancy rate and determination of fetal number at 45 dPB). Therefore, the effective size of the first-time bred goats included in the analysis was 446.

Reproductive performance

For the independent variables of primary interest (*i.e.,* P4 exposure, breeding procedure and PG600 dose level), the distribution of the 879 goat breedings used in the study, which were either the result of a goat being diagnosed pregnant or open as well as goats not scanned, is given in Figures 6 through 8. As detailed in Figures 6 through 8 a total of 205 records correspond to breedings that were not subject to the scheduled 45 dPB UI due to non-anticipated scan scheduling omissions or inadvertent oversight in different breeding groups for various reasons. Nonetheless, 30% (61/205) of the bred goats that were not scanned for pregnancy diagnosis or were scanned at an inappropriate time eventually kidded (1 aborted).

The ultrasound pregnancy data was generated from a total of 674 evaluations of which 142 (21%) records were invalidated and not used; 21 goats were scan-evaluated too early (<35 days) and the remaining 121 observations were from goats with ultrasound scans that took place late (>55 days). Hence, the effective sample size was 530 ultrasound observations. The excluded records correspond to breedings that eventually had a PR of 56% (80/142; with 4 abortions).

Conception rate (CR)

Overall herd CR was 1.7 newborns per goat and, in terms of does conceiving, this represents a 57% CR (Table 2).

When CR was analyzed as a function of breeding number there was a significant difference ($P<0.0002$) attributable to the different breeding sequence number. However, the inclusion of records generated on the 4^{th} breeding was deemed improper due to its

small sample size which triggered a concomitant increase in heteroscedasticity (i.e., invalidating the ANOVA). This increasing variance effect, present from the first to the 4th breeding, can be clearly observed on panel [A] of Figure 9. Nevertheless, results from formal variance homogeneity tests were ambiguous with an O'Brien and a Brown-Forsythe test indicating uniform variances ($P>0.2164$) and ($P>0.10$), respectively and a Levene's test rejecting that the variances were homogeneous ($P<0.008$).

We chose to be conservative and did not include, in the analysis of CR, results from the 4th breeding. Using the most basic model, where the main effect is given by breeding number (from first to third) the statistical influence of breeding number on CR was not modified ($P<0.0001$) and remained as observed in panel [A]. Panel [B] shows the number of breedings used, with this arrangement all three variance homogeneity tests mentioned above indicate that the variability associated with each CR mean was constant across breedings ($P>0.05$).

Mean separation (Tukey HSD; Q) indicates that the first breeding had a different CR than the second or third subsequent breedings (n= 872). This calculated significance is portrayed in Table 3, where levels not connected by same letter represent means that are significantly different.

When the CR statistic is analyzed exclusively on the basis of UI diagnosis at 45 dPB, it is no different than PR. However, if CR is evaluated with all the direct and indirect information available (*i.e.,* 25 d non-return rates, presence of embryo(s) at 46 dPB ultrasound scan, and atypical estrous cycles lengths) as shown in Figure 10, there is a marked improvement in the resulting calculation. That is, the overall CR was estimated to

be 72% (n= 879) and the first breeding 67% (n= 533). In each case estimated CR was improved by approximately 8% percent units by using atypical estrous expression.

A 61% CR was determined for the 533 first-breeding goats when the calculation excluded atypical estrous length as the cue for goats having conceived and considered fertile goats on the basis of prenatal losses as depicted in Table 23. No difference ($P>0.086$; OR=1.2) was detected between overall data CR and that calculated for the first-breeding goats.

Pregnancy rate (PR)

As compiled in Table 4, the overall herd PR for the duration of the project, for goats evaluated by ultrasound at 46 dPB, was 59% (398/674). The 55% PR (247/446) for the first breeding was 12% points less ($P<0.007$) than the cumulative PR for untreated, non-synchronized, goats (*i.e.,* other than the first breeding attempt) bred up to a 4th opportunity by non-fixed time NS which was 67% (151/228).

In Table 4 the first-breeding attempt includes all three types of fixed-time breeding procedures (*i.e.,* NSp, TrAI and LAI) as well as all synchronization protocols considered in this study (including both control groups [N and NNT]; see Figure 3), whereas breeding attempts two, three and four, were performed only on spontaneous estrus (no estrus synchronization protocol) and only non-fixed-time NSp was used.

Overall pregnancy rate of the first breeding was influenced ($P<0.0001$) by P4 protocols used to synchronize estrus and ovulation as presented in Table 5. Fixed-time NSp (excluding the NNT treatment group which was not synchronized and time-bred) resulted in the highest PR with 61% (133/220) compared with the 46% PR for fixed-timed LAI

(P<0.0154) or the latter compared to the 27% PR attained with fixed-time TrAI (P<0.0001).

Kidding Rate (KR)

As seen in Table 6 the overall KR has a range of 52 % points depending on the criteria used to calculate the statistic. The global KR for the first fixed-time breeding based on mated goats shows a difference of 29 percent points (P<0.052; χ^2=3.783) when compared with a KR calculated on the basis of fertile females. These values result in an OR of 0.696 with a $CI_{95\%}$=(0.49, 0.99);

Fixed-time breeding KR for goats mated was 46% (see Table 7, last column) and was most similar to a 44 % KR calculated in Table 6 on the basis of does mated (which represents the most common manner of evaluating KR). The KR for non-synchronized breedings (*i.e.,* 2, 3, and 4) was 40% (137/346) which turned out to be less (P<0.06) than the 46% (246/533) KR for synchronized goats.

As given in Table 8, the overall 60% (109/181) KR for the first-time mated goats not synchronized was analyzed by OR and point to a first-breeding being 2.4× more likely to result in a greater KR percentage than goats bred on a synchronized estrus which yielded a 40% KR (P< 0.0001). This KR value compares unfavorably to a similar KR calculated on the basis of fertile goats which came out to be 86.5% (109/126); see Table 9.

The fertility rate according to the breeding procedure used in the first fix-time attempt shows that goats bred by natural service have greater fertility than LAI being 3× and 4.2× more likely of having a greater KR than did LAI or TrAI (P<0.00001), respectively.

There was no significant difference observed between TrAI (23%) and LAI (30%) with the latter 7 percent points higher and an OR of 1.4× (P >0.30).

Fertility (F)

The breeding group fertility based on first fixed-time breeding (*i.e.,* does mated) was 61% (326/533) regardless whether goats were synchronized or not. As shown in Table 10, the proportion of goats kidding in relation to the number of fertile goats was 76% (246/326).

When no adjustment is made for prenatal losses, F is 44% at this level of fertility there is a difference (P<0.001) between PR and F. Whereas, no significant difference (P>0.58) was found between the overall (59%) PR based on UI data and the (58%) F based on actual kidding outcome when kidding was adjusted for actual fertility condition. That is by considering that goats which had prenatal death losses were in fact fertile.

When the 54% F of synchronized goats is compared with the 46% F of non-synchronized goats the 8% unit difference and OR= 1.4 becomes significant (P<0.02), likewise, when the same comparison is made considering only fertile goats, the absolute rate increases for both synchronized and non-synchronized groups. However, the 8% unit difference and OR= 1.4 remained unchanged even if the significance of the comparison changed somewhat (P<0.03) between the 61% F for synchronized goats compared to the 53% F of the non-synchronized.

As portrayed in Table 11, fertility of first fixed-time breeding was highest for NSp (67%), followed by LAI (54%) and TrAI (42%). F of the NSp group was different from LAI (P<0.011) and TrAI (P<0.00001). LAI and TrAI did not differ (P>0.09) on their F levels associated with these breeding procedures.

NSp was 2.9× and 1.8× more likely of yielding a greater fertility than TrAI or LAI, respectively. Goats that were not EO/S had greater (P<0.0003) F (72 %) than goats that were EO/S (55%). Non-synchronized females were 2× more likely of yielding a greater F than synchronized goats.

Prolificacy (Pr) and fecundity (Fc)

Prolificacy and fecundity are two interrelated RETs which, in both cases, evaluate the number of kids born alive as a function of two different categories of does. That is, when the number of progeny is related to does mated the coefficient is known as prolificacy (Pr) and when the number of progeny is related to females that kidded then it is called fecundity (Fc). Therefore, for ease of comparing Fc and Pr, results are simultaneously presented for both parameters.

Overall Pr and Fc were calculated to be 1.8 and 1.01 live kids, per mated doe or per kidding goat, respectively. As tabulated in Table 12, Pr and Fc for the first fixed-time breeding were 1.8 and 0.83, respectively. On one hand, there were no differences (P> 0.983) for Pr, in regards to the type of procedure used for breeding. Both LAI and NS had a Pr of 1.81 and TrAI reached a level of 1.79 kids born alive per goat that kidded.

Fecundity, on the other hand, was highly influenced by type of breeding (P< 0.0001). NSp resulted in the highest Fc with 1.01 kids born alive per mated goat. Breeding by NSp was 60× and 124× more likely to have a greater Fc than when goats were bred by LAI or TrAI, respectively. The Fc of LAI and TrAI was 0.54 and 0.36, respectively. The difference between these two procedures was also significant (P<0.017) with goats bred by LAI 2× more likely to have high fecundity than goats bred by TrAI.

The effect of breed and breeding number on goat prolificacy and fecundity

Because of its known influence on Pr and Fc, breed was analyzed as a function of breeding number. The resulting quantitative composition of comparison breed groups is given in Table 13. As a percentage of total goat number the x-bred goats (39%) were the phenotype most represented while the Spanish breed were the least (7%). Also see Figure 11; panel [A]). Eighty percent of the goat population was bred by the second estrus (Figure 11; panel [B]).

As shown in Table 14 the overall herd Pr was 1.8 kids born alive per doe bred (see also Figures 12 and 13) and Fc reached 101% (*i.e.,* an average of 1.1 kids born alive per mated goat; see also Figures 12 and 13). Overall breed influenced both goat Pr and Fc ($P<0.0001$) while breeding sequence number did not have an effect on Pr ($P>0.72$) but it did influence Fc ($P<0.02$).

As seen in Table 15, Pr was not influenced ($P>0.541$) by the number of times a goat was bred. Graphically, however, (see Figure 12) a tendency for a lower Pr to be associated with the number of times a goat was bred is noticeable with a drop in Pr particularly noticeable in the last breeding.

Prolificacy was greatest for Boer (1.9) and least for the Angora (1.2) breeds. The LSM separation (see Table 16), where levels not connected by same letter are significantly different, indicates that the Angora breed differed from all other breeds ($P<0.0009$). All other breeds did not differ amongst themselves ($P>0.05$).

Breeding number influenced Fc ($P< 0.0207$). And, as shown in Table 17, the 4^{th} breeding turned out to produce similar Fc as that found in the first and third breedings. The second

breeding was similar to the third breeding but different to the first and fourth breedings. The fourth breeding had actually 1.5× better Fc than the mean average fecundity of all four breedings (see also Figure 13).

Data analyzed shows that breed influenced Fc ($P<0.0172$). Fecundity was greatest for Spanish goats (1.2) and least for Tennessee Stiff Leg (0.3). The LSM separation (see Table 18), where levels not connected by same letter are significantly different, indicates that the Spanish breed differed from all other breeds. As portrayed in Figure 14, the Spanish breed had more the 3× the level of Fc than the average Fc of the two lowest breeds. The Boer, Alpine and x-breds had similar Fc while expressing almost twice as much Fc than the Angora and Tennessee Stiff Leg breeds. These latter two breeds had similar Fc among themselves.

Logistic regression models for analysis of main treatment effects on reproductive efficiency traits (RET).

Using diverse combinations of the independent variables (*i.e.,* P4 protocol, breeding procedure, use of PG600, breed, age [continuous] or age groups [ordinal], parity, year of breeding), and relevant interactions, various statistical multiple logistic regression models, were fitted to the RET data generated from UI (n= 446), attrition losses during gestation and kidding at term resulting from pregnancies to the first-breeding.

Starting with a partially saturated model [1],

[1] **Log(n) E(Y)=** $ß_o + ß_1 \times$ P4 Protocol $+ ß_2 \times$ Breeding procedure $+ ß_3 \times$ PG600 $+ ß_4 \times$ Parity + AI Tech $+ ß_5 \times$ Breed $+ ß_6 \times$ Age group $+ ß_7 \times$ Breeding year + ($ß_3$ PG600 $\times$ $ß_4$ Parity)

where,

E(Y) represents the expected log value of the odds ratio given by the probability (P) at first-time breeding of a goat having been diagnosed as compliant with conditions for a positive response (*i.e.,* pregnant or number of embryos) compared to that of a goat being diagnosed open (1-P). ßo represents the intercept of the linear model and $ß_1$ through $ß_7$ represent the coefficients of the equation regressors.

Independent variables were sequentially removed from the model when they did not improve model fitness and/or the variables of interest lacked statistical significance. The structure composition of the final streamlined reduced model [2] chosen,

[2] **Log(n) E(Y)=** ßo + $ß_1$× P4 protocol + $ß_2$× Breeding procedure + $ß_3$×PG600 + $ß_4$×Parity + $ß_5$ × Breed + $ß_6$ ×Age group

was consistent across RETs and only varied once in that the response variable "fecundity" (bottom panel of Figure 15) was not influenced by the age group in which a goat was categorized. Although PG600 provided no statistical significant influence for any of the RETs considered, being one of the three central explanatory variables (main effect) in this study, it was included in the model for completeness illustrative purposes. Excluding this PG600 from the statistical analysis of the logistic model did not modify any of the inferences arrived at.

Except for Pr and Fc (P for χ^2 >0.95), the best reduced logistic model chosen, although with great significance (omnibus test P<0.0001), fitted poorly to the remaining RET data; goodness of fit (P for χ^2 <0.001). After fitting the full model with data generated with these six regressors, the –Log Likelihood showed a decrease for the full model from the

reduced model that varied with the RET analyzed. The ratio of the difference from the full to reduced model represents the proportion of the uncertainty attributed to the fit of the model chosen and reported as the R^2 of uncertainty (U). That is, CR=18%, PR= 23%, F= 12%, KR= 18%, Pr= 19%, and Fc= 14%.

Effect of E/OS protocol on reproductive efficiency traits

The protocol used to synchronize estrus and ovulation differed in the time each treatment group of animals were exposed to P4 (*i.e.,* period of time of CIDR retention) and the dosage of PG600 given. As can be seen in all the panels of Figure 15, PG600 had no influence on all the RETs. Consequently, significant synchronization effects observed, as a result of protocol, are associated only with the P4 time exposure used.

All RETs were influenced (from $P<0.001$ to $P<0.0001$) by the time that CIDR's were in the reproductive tract. Holding other RET at their average values, goats in the XL P4 exposure treatment had 5 to 40× greater odds of conceiving than other goats on different P4 exposure treatments. This represents 83% to 97% greater probability of conception.

As can be evidenced in Figure 15, CR, PR and KR had very similar patterns of influence with XL- P4 giving the highest OR's and NNT (no P4 and no fixed-time breeding) the lowest OR values. Pr and Fc also show similar patters across embryo numbers detected, but goats not synchronized and not fixed-time bred generated the highest absolute values.

Effect of breeding procedure on reproductive efficiency traits

As seen in Figure 15, the breeding procedure used had major influential effects on all 6 RETs studied: CR ($P<0.044$), Pr ($P<0.025$), and for the rest of the variables ($P<0.001$). As tabulated in Tables 19, 20 and 21, within each breeding procedure, PRs were

influenced by the type of P4 protocol used. The interaction effect between P4 protocol × breeding procedures on RETs could not be tested on the complete first-breeding data base due to the absence of several data cells. The lack of information generated a nominal logistic regression model platform with, singularities, biased and zeroed parameter estimates due to linear dependencies in the design. Interactions were verified (data not shown) using a model which included data only for P4 protocol and breeding procedure (n= 394). Interactions were present for CR (<0.0001), PR (P<0.05), F (P<0.0178) and KR (P<0.009). Pr and Fc were not affected by P4 protocol × breeding procedure interaction; (P>0.082) and (P>0.267), respectively.

In general, the 6 d short exposure to P4 resulted in less or equal PR (P<0.01) than the 12-14 d longer periods of P4 exposure or when no P4 was used and the animal was bred on a natural estrus occurrence. Extreme low PRs were found when P4 exposure was extra-long (24 d).

Natural Service. Overall results indicate that a goat bred by means of NSp (irrespective of estrus synchronization protocol) will be 2.8x (P<0.001) more likely to become fertilized than if bred by LAI and 4.4x more likely to be fertilized (P<0.001) than if bred by TrAI. Although a numerical difference of 11% units was found between the fertility obtained by use of LAI (41%) and that attained using TrAI (30%), there was no statistical difference (P>0.136) between both breeding technologies.

The NNT treatment had the highest PR at 86% and was significantly different (P<0.001) from all other synchronized treatments. CR for L/SynB (83%) was not different from that attained by the L (71%) treatment and practically the same as the CR obtained by NNT

(82%). F was greatest for L/SynB (72%) and least for XL P4 (20%). Pr was only different between the L (2.2) and the NNT (P<0.034). F was different from all comparisons made except for that obtained between NNT and N (P>0.538). KR was greatest (100%) for goats bred at a fixed-time but using a natural occurring estrus and lowest (63%) for goats under the XL P4 treatment.

The highest PR (for fix-timed breedings) was obtained when breeding was performed means of NSp using a long P4 protocol (12 to 16 days) regardless of the manner in which P4 was delivered (*i.e.,* intravaginally or as an ear implant). Both long P4 protocols gave the same PR as that obtained with goats where there was no estrus synchronization.

When using fixed-time NSp both extremes of P4 exposure gave the lowest PR; 50% for the short P4 protocol and 26% for the extra-long progesterone protocol. On average, the long and no-treatment P4 protocols were about 7.5 times more likely (P<0.00001) to result in a pregnant doe when compared with the short and extra-long P4 protocols, respectively.

The only case where the short P4 protocol gave better pregnancy rates was when compared to the extra-long P4 protocol where the probability of having a doe pregnant was 2.8 times better with the short P4 treatment rather than the extra-long P4 protocol.

Laparoscopic AI. Conception rate differences (P<0.027) were documented only between the long (55%) and short (45%) P4 protocol.

When using intrauterine insemination by laparoscopic procedures the odds that a doe will become pregnant is about 5 times greater (P< 0.01) if estrus has been synchronized with the long P4 protocol or if LAI is performed on a natural estrus rather than when P4 is

used for 6 days. The PR resultant from goats that were synchronized for 12 to 16 days was not different (P>0.115) compared to the PR obtained when goats were bred laparoscopically on a non-synchronized estrus.

As presented in Table 20, CR (P<0.027) and KR (P<0.004) were influenced only by the difference in P4 length of exposure associated between the L and S P4 protocols. Both F and Pr were not influenced (from P>0.084 to P>0.724) by any of the P4 protocols. In contrast, Fc (see Figure 16) was the RET most influenced by length of P4 used when synchronizing goats (P<0.0126).

Transcervical AI. Shown in Table 21 is the 63% CR (P<0.002), 2.1 Pr (P<0.03) and 88% KR (P<0.03) generated by the S synchronization protocol were different than the 36% CR, 1.0 Pr and 42% KR of control (non-synchronized) goats. The S P4 protocol again had favorable influence over CR (P<0.055) and Fc (P<0.003) when this protocol was compared with the 20% CR and 16% Fc of the L P4 administration.

Effect of gonadotro*pins (PG600) dosage level on reproductive efficiency traits*

The evaluation and treatment means comparison performed on the effect of PG600 on RET was based on 427 (80%) first-time breeding goats that received exogenous gonadotropins, as shown in Table 22, and a total of 106 (20%) untreated goats which served as a control group.

Because in the goat exogenous eCG has a FSH-like biological effect, *a-priori,* suspect reproductive parameters subject of being influenced by its action, are ovulation rate (not measured in this study) and variables directly correlated to the number of ova produced such as the number of embryos produced (evaluated through CR or Pr or Fc). Likewise, attainment of high levels of any of these RETs followed by a discrepancy or by a severely

unmatched litter size at kidding may be indicative of heavy prenatal losses potentially influenced by hormone protocols used for E/OS. Therefore, the evaluation of PG600 effects on RETs centered exclusively on both the number of embryos at conception and litter size at kidding.

Embryo number at conception. The overall first service CR was 61% with an average prolificacy of 1.1 and 1.8 kids per mated or artificially inseminated female and per kidding doe, respectively. As presented on Table 23, first service CR was highest (66%) for goats that did not receive PG600 compared with 60% CR for goats receiving 5 mL and 55% CR for goats that were treated with 1.75 mL of PG600. However, only the difference of 11% units between the non-treated goats and those animals that received 1.75 mL of PG600 was significant ($P<0.02$).

Effect of breed and age group on reproductive efficiency traits (RETs).

Breed and the age category group to which goats were assigned to were included in the logistic regression model as blocking variables to evaluate main treatment effects (*i.e.,* P4 protocol, breeding procedure, and PG600 dosage). Both covariates contributed differently to the overall model's significance depending on the RET'S involved in the analysis.

The Tennessee Stiff Leg breed had almost 16×, the Angora breed had 10× and the Boer breed 9× the likelihood of being diagnosed as having conceived by 46 dPB than Spanish goats. Mature goats, greater than 5 y old, were 5× more likely than goats of age 4 to 5 y to conceive and, this latter age group was 2.7× less likely of being detected as having conceived by 46 dPB.

Angora goats were 77×, 24× and 14× more likely to be pregnant than open compared to Spanish, Boer and Alpine goats, respectively. Similarly, the older group of goats was almost 5× more likely to be pregnant compared to goats of age 4 to 5 y old.

Tennessee Stiff Legs were almost 5× and 6.5× more likely to be fertile than Alpine or Spanish goats. Goats of age older than 5 y of age were 2× more likely to be fertile than the younger 4 to 5 y-olds.

Angora goats were almost 9× more likely to kid than the Alpine breed. Whereas Stiff-Legs were 6× more likely to kid than Spanish goats were. With regard to age, grouped older does (>5 y) were 3× and 2× more likely to kid than the 4 to 5 age group and the group of 3 to 4 y-olds, respectively.

Breed was close to statistical significance ($P<0.058$) in influencing Pr and definitively influenced Fc ($P<0.003$). Age of goat influenced Pr ($P<0.03$) but not Fc ($P>0.05$).

Discussion

For purposes of assessing global herd reproductive accomplishment, overall RP of six goat breeds representative of dairy (Alpine), meat (Boer , Tennessee Stiff Legs and various genotypic percentage Boer x Spanish crosses) and fiber (Spanish/Cashmere and Angora/mohair) phenotypes was evaluated. In terms of age, the range of 1.5 to 11 y covers a wide group and the ages considered are deemed to reflect similar herd composition of what most goat producers would have.

Characterization of female RP requires considering peri-estrus/ovulatory events throughout gestation to kidding. Hence, representative traits of important reproductive landmark measures were chosen. Therefore, six reproductive efficiency traits (RET), which cover the span of time from conception to kidding at term were used for this assessment (*i.e.,* CR, PR, KR, F, Pr, and Fc). Two important early reproductive parameters (ovulation rate and fertilization rate) were not considered in this study. Available evidence indicate that goats ovulate an average of 2.64 ±0.40 ova and of these released oocytes an average of 62 ± 23 % become fertilized when breeding takes place under natural conditions.[51] In sheep, fertilization rate, determined 72 h after AI, was greater ($P<0.05$) after laparoscopic than after transcervical/cervical AI (92.5 vs 28%).[52]

Since PR was determined on the basis of UI it was deemed a pre-requisite to establish the validity of this technology in our hands. The results of such analysis are presented in Chapter III of this dissertation. In brief, the validity of the ultrasound procedure was confirmed for diagnosing reproductive status (i.e., pregnant or not pregnant) but predicting the number of fetuses was influenced by both productive phenotype and parity making PR's solely dependent on UI at 45 dPB unreliable.

After verifying the absence of a statistically significant seasonal effect (i.e., breeding year effect) on the seasonal accumulated RET data,; tests where the null hypothesis is that of no statistically significant difference: CR ($P>0.28$), PR ($P>0.35$), F ($P>0.40$), KR ($P>0.12$), Pr ($P>0.11$), and Fc ($P>0.13$), all the data was coalesced into one database.

Much of the reproductive comparisons of interest, between the results obtained in this study and other results is limited due to the scarcity of literature available on the germane subject. The difficulty concerns the validity of comparisons due to: a) non-standard E/OS protocols used in evaluating RP, b) results were not pursuant to an *a priori* formulated hypothesis but a by-product result of research with different objectives, c) focus is on E/OS protocols that either studied individual effects of eCG or hCG. d) reproductive performance analysis has centered on one efficiency measure; that being pregnancy rate which only describes one of the events necessary to understand reproductive performance holistically and, e) parameterization of RETs is not consistent. That is, reproductive parameters are calculated with mathematical formulae that incorporate different components.

Few published reports address the concomitant use of eCG and hCG in small ruminants.[1] Research has established that lactating goats (≥120 DIM) using norgestomet implants for 9 and 12 d during the transitional phase had no significant association between does treated with the hCG/eCG combination and goats treated with reagent-grade eCG, regarding the percentage of does coming into estrus (89 and 97%, respectively).[53] These same authors found however, a significant difference for pregnancy rate when goats were bred by natural service (90% vs 76% for hCG/eCG and eCG treatment groups, respectively). The estrus and ovarian response to different doses of PG600 (0, 80, 160,

320, 640, and 1,280 IU) at the time of implant removal following 12 d norgestomet provision in both breeding and anestrous season was evaluated.[54] Estrus response and time to estrus were not affected by dose level, but CL number increased with dosage increases in both seasons.

The effects of melengestrol acetate (MGA) and PG600 on Rambouillet ewe fertility outside the natural breeding season were evaluated. Although PG600 increased the number of luteal structures present per ewe, it did not significantly enhance ewe prolificacy.[55] In a different study during the breeding season, the feasibility of timed AI was evaluated in hair sheep ewes and meat goat does using medroxyprogesterone acetate sponges for 14 days (sheep) and 16 days (goats), PG600 was given at time of sponge removal. Animals were laparoscopically inseminated and pregnancy rates were 0% in goats and 8% (1/12) in sheep.[17]

Melengestrol acetate (MGA) and Syncro-Mate-B (SMB), were evaluated for their ability to induce synchronized estrus in anovulatory ewes. Fertility and prolificacy were not different for treated ewes. Ewes primed with Zeranol before MGA or SMB treatment had fertility and intervals from ram introduction to lambing similar to those of ewes receiving an injection of PG600 after progestogen treatment.

Reproductive Performance

Conception Rate

Conception rate has not been evaluated in goats. Some published information uses PR as a synonymous of CR.[56-58] In this study we estimated a 67% CR for first- time breeding and 72% overall CR.

Because the sole difference between the first breedings and the rest of the breedings is that hormonal synchronization was used on some treatments of goats bred the first time the lack of difference (P>0.086) detected between the CR of the overall data and that of the first-breeding goats it implies that the E/OS protocol used did not influence CR.

Some studies in sheep and cows suggest that the levels obtained in this study are within what would be expected when the evaluation is made in non-synchronized and non-fixed time bred goats. For example, the CR's of synchronized goats (no fixed-time breeding) was 58.6% when semen placement was deep in the cervix or intra-uterus compared to 39.5% when semen was deposited at the entrance of the cervix.[59]

Pregnancy Rate

The 59% overall pregnancy rate determined in this study (n= 674) at 46 ±4 dPB is lower than other reports where PR for sheep was from 50 to 93%.[79] However, the global results are strongly influenced by the various synchronization and breeding procedure treatments imposed on goats. For example, for naturally serviced, non-treated goats (*i.e.,* non-synchronized and non-fixed-time bred) the PR was 86% for the first breeding.

Other studies have not been performed to determine the effect of fixed-time breeding on non-synchronized goats that are bred using natural service. In this study fixed-time breeding alone depressed PR by 24 percent units (PU). This difference decreased to 12 PU when compared with the 74% PR of the long P4 protocol. Extreme differences of 29 and 66 PU were observed when animals were treated either with a short or an extra-long P4 protocol, respectively.

The global comparison of fixed-time NSp (excluding the NNT treatment group which was not synchronized and time-bred) which resulted in the highest PR with 61% compared with the 46% PR for fixed-timed LAI ($P<0.0154$) or the latter compared to the 27% PR attained with fixed-time TrAI ($P<0.0001$) implies that one can expect fixed-time NSp to be 4.2, and 1.8× more likely to result in a pregnancy than TrAI or LAI, respectively. Likewise, LAI is expected to be 2× more likely to result in a pregnancy than if a goat is bred by fixed time TrAI.

Laparoscopically attained fixed-time breeding PR of 71% was the greatest attained for artificially bred goats and was not different from the average of goats naturally serviced in the long P4 protocol (CIDR and Synchromate B) or control groups (N and NNT). In other studies (Castilho *et al.,* 2010) laparoscopically inseminated sheep with ewes given eCG or EB had a PR of 67% and 11% respectively.

The PR of goats artificially inseminated by transcervical means was consistently low at 26% regardless of the E/OS protocol used. Similar PR (24%) was obtained with the S protocol of LAI.

Sönmez et al., (2009)[58] found an overall KR of 63% (with no differences between 59% in control goats and 67% for goats receiving vitamin E).

Overall PR for goats treated with intravaginal 60 mg medroxyprogesterone acetate sponges for 9 d plus 200 IU eCG and 22.5 µg d-cloprostenol 24 h before sponge removal was 60.7% (65/107) where nulliparous had 60.0%, non-lactating 44.4% and lactating 77.8%.[56]

In this study the treatments labeled as "trans-cervical" describes the breeding procedure attempted but does not imply that the breeding was necessarily accomplished by traversing of the cervix. The reason of this low PR could be due to a high proportion of females being bred in the vagina vestibule rather than in the cervix or in the uterus proper. As it has been shown in other studies, fertility may be markedly lower for cervical insemination than for intrauterine insemination.[40, 60]

In goats, there are reports that the progestagen treatment decreased PR and KR, especially if the animals are in the breeding season.[61] While other researchers have found no differences between progestagen treatments.[62, 63]

Kidding Rate

In this study KR's ranged from 44% to 96% depending on how the rate was calculated. Probably the most comparable KR is the one calculated as a function of the number of does mated, in which case the KR was 44% (Table 6). Likewise, the overall KR to fertile breedings was 58% (Table 10). These very different KR results (5 in total) point to the fact that this statistic needs to be exactly defined if it is going to be compared across studies. The 44% KR obtained is lower than what has been obtained in other research where an overall KR of 51.4% was found (with no differences between 56% in control goats and 47% for goats receiving vitamin E).[64]

Fertility

The overall fertility attained was 58% and fertility to the first breeding was 61%. Breeding procedure influenced fertility rates with 42% for TrAI, 54% for LAI and 67% for NS. Control animals that were bred by NS with no synchronization and no fixed-time

breeding attained high fertility at 85% although goats in the same group receiving 12-14 day P4 exposure reached the highest fertility at 93%.

Prolificacy

In this study we found that prolificacy ranged from an average of 1.0 for goats in the S exposure P4 protocol bred by TrAI and 2.2 kids per kidding female in the naturally bred LSyn/B group receiving P4 ear implants for 12-14 d.

When goats were not synchronized (both control N and NNT groups; average of 1.65 Pr) and were bred by NS the pattern observed is as expected one where does treated with gonadotropins have an increased hormonal stimulus to produce more kids. Nevertheless, in this study the only significant difference documented was that between the NNT (1.7 Pr) and the LSyn/B (2.2 Pr) and that between N and S.

The 2.0 prolificacy obtained in this study for goats treated for 12-14 d compares favorably with the prolificacy found in other studies. A prolificacy of 2.40 ± 0.37 was found for goats receiving a dose of vitamin E and 1.63 ± 0.26 kids per kidding for goats not treated with the vitamin.[58] In the latter study estrus was synchronized in 36 non-lactating adult does using intravaginal sponges containing 30 mg of fluorogestane acetate (FGA) for 14 d. All females received 500 IU of eCG at the sponge withdrawal.

Fecundity

In this study Fc for synchronized, fixed-time bred goats was 1.09 compared with the control (no synchronization and no fixed-time breeding) group of goats which had 0.89 ($P<0.0001$). Other research in goats bred by natural service and comparing the use of progestagen laden vaginal sponges to synchronization with two doses of $PGF_{2\alpha}$ or to the

controls with no synchronization[65] fecundity rates were 2.15, 1.75 and 1.80 for each group, respectively, with a significant difference ($p < 0.05$) between the sponges and both $PGF_{2\alpha}$ and control groups.

Spanish goats turned out to be the most fecund with a coefficient of 1.21, this level of Fc was different from all other breeds ($P<0.05$). Boer, Alpine and x-bred goats were similar ($P>0.05$) with $F\approx0.81$. Angora and Stiff-leg goats had the lowest Fc, 0.45 and 0.30, respectively.

Chorionic gonadotropins (eCG and hCG)

PG600 was used as the source of eCG and hCG. In this study we did not find influence of any of the two levels of PG600 used on any of the RETs. Consequently, effects observed which may be a result of E/OS protocol, are associated with the P4 time exposure used.

Breeding procedure

As expected animals bred by natural service with no treatments applied resulted with the best RP. AI generated the lower RETs with TrAI having the lowest levels. Noteworthy was the effect of fixed-time breeding which was shown to reduce RETs even when using NS beyond the negative effect of the E/OS protocol or type of breeding procedure.

Conclusion

The use of hormonal E/OS protocols combined with fixed-time breeding appears to have compounded negative effects on reproductive performance. The low CR, PR and KR seemed to be connected to the possibility that synchronization protocols could be influencing the time of ovulation, therefore the appropriate time of insemination. However, this explanation is not consistent with the high CR, PR and KR obtained with LAI which used the same time period to deliver semen.

Anecdotal evidence gather in the early stages of this study pointed to the apparent possibility that goats receiving less P4 exposure were more difficult to inseminate by TrAI. Research crossing many scientific disciplines has demonstrated that P4 also affects the cervix through a myriad of cytoplasmic and membrane receptors.[66] The effect of P4 can also occur by way of prostaglandin E2 when it binds to its cognate receptors stimulating hyaluronan synthesis. The glycosaminoglycan hyaluronan is one of the chief components of the cervical extracellular matrix. The ability of this carbohydrate polymer to imbibe water may culminate in cervical relaxation[67] and/or, by the effects as related to rapid or poor accessibility of the cervix at time of AI, on plasma cortisol and oxytocin, and uterine motility.[68]

From the results obtained, further studies looking at the effect of the E/OS protocols used with regard to breeding difficulty are recommended. Particularly because when breeding is done by-passing the cervix (when using LAI) much higher RP results are generated. This putative detrimental effect on the cervix may increase the time of breeding and be counterproductive by added constraints placed on the goat's reproductive anatomy such as when the AI gun tries to traverse an incompletely relaxed cervix. Corroborating this

possibility is the suggestion that rapid semen deposition limits reflex uterine contractions provoked by the speculum and the movement of the insemination gun, negatively influencing reproductive performance to first AI in nulliparous goats.[69] Indeed, at least one other recent review brings together research findings on cervical relaxation in the ewe and its pharmacological stimulation for enhancement of the penetration needed for transcervical insemination.[70]

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Table 1. Breeding records by synchronization protocol, breeding procedure and number.

		No fixed-time breeding		Fixed-time breeding						
		Control				Short	Long		Extra-long	
		Not synchronized				CIDR	CIDR	Synch B	CIDR	
	Total Nº of breedings ▶	1	2-3-4	1	2-3	1	1	1	1	TOTAL
Breeding procedure	LAI[a]	--	---	25	4	49	333	--	--	111
	NSp[b]	85	332	49	--	68	93	29	25	682
	TrAI[c]	--	---	22	10	30	25	--	--	87
	Grand total	85	332	96	14	147	151	29	25	**879**
					533[d]					

[a] LAI, laparoscopic artificial insemination.
[b] NSp, natural service by pen breeding.
[c] TrAI, transcervical artificial insemination.
[d] Total of goats bred once.

Table 2. Overall conception rate based on: a) 46 dPB ultrasound detected number of embryos, and b) on does conceiving as a function of breeding number.

		Nº of embryos detected by 46 dPB-ultrasound						Conception rate based on number of embryos		Conception rate based on maternal conception	
Breeding Nº	**Goats bred**	**0**	**1**	**2**	**3**	**F[a]**	**Not scanned**	**Total E embryos**	**CR-E[b] (%)**	**Nº goats conceived**	**CR-M[c] (%)**
1	**533**	198	71	308	54	3	87	436	1.76	324	61
2	**272**	65	55	126	3	3	84	187	1.52	139	51
3	**67**	10	13	24	--	--	32	37	1.48	34	51
4	**7**	2	--	6	--	--	2	6	2.00	7	100
Total	879	275	139	464	57	6	205	666	1.67	504	57

[a] Fluid detected; usually found at 20 to 35 days post breeding, hence indicating a later breeding.

[b] Conception rate based on the number of embryos attached per fertilized female (based on 46d PB ultrasound).

[c] Conception rate based on maternal conception; number of does with attached embryos per number of does bred.

Table 3. Conception rate (CR); least square means (LSM).

Level	CR-E[a] LSM		CR-M[b] LSM	
	(P<0.0001)	Q[c]= 2.35	(P<0.171)	Q= 2.35
1	A	1.78	A	62%
2	B	1.55	B	53%
3	B	1.48	A B	48%

[a]CR-E: Conception rate based on embryos detected by ultrasound.

[b]CR-M: Conception rate based on maternal conception.

[c]Q: Mean separation based on Tukey's HSD.

Table 4. Overall pregnancy rate[a] by breeding number.

	Pregnant		Open		Subtotal		Not Scanned		Total	
Breeding Nº	Nº	% [d]	Nº	%	Nº	Cumulative%	Nº	% [e]	Nº	%
1[b,c]	247	55	199	45	446	66	87	17	533	100
2[f]	123	65	65	35	188	94	84	31	272	100
3[f]	25	60	10	24	35	99	32	48	67	100
4[f]	3	75	2	50	5	100	2	29	7	100
Total	398	59	276	41	674	100	205	20	879	100

[a] Pregnancy evaluated at 46±4 dPB by ultrasound scan.
[b] Includes all three methods of timed breeding (NSp, TrAI, LAI).
[c] Includes all methods of hormonal synchrony (S, L and XL) and goats not synchronized.
[d] Percentage calculated on the basis of total goats ultrasound scanned per breeding group.
[e] Percentage calculated on the basis of total goats per breeding group.
[f] Breedings with no estrus synchronization and using only NNT (NSp non- fixed-time bred).

Table 5. Pregnancy rates: first breeding by breeding procedure and P4 exposure time.

		Intrauterine (LAI)			Natural Service (pen)						Transcervical (TrAI)			
Progesterone (P4) protocol		L	N	S	L	L SynB	N	NNT	S	XL	L	N	S	Grand Total
Pregnant	Nº	20	17	12	39	24	28	48	37	5	6	6	5	247
	%	61	71	24	70	83	62	86	57	20	26	27	26	55
Open	Nº	13	7	37	17	5	17	8	28	20	17	16	14	199
	%	39	29	76	30	17	38	14	43	80	74	73	74	45
Total number		33	24	49	56	29	45	56	65	25	23	22	19	446
Average pregnancy		46%			74%		75%				27%			
					66%									

Table 6. Different ways of calculating kidding rate.

When the criteria for comparison is:	Kidding		Mean comparisons			
	(Nº)	(%)	Different letters within columns indicate significant differences			
Breeding group	879	43.6	A			
Does mated	867	44.2	A	A		
Fertile does	511	75.0	B	B	A	
Does conceiving	504	76.0	B	B	A	A
Goats pregnant	398	96.2	B	B	B	B
Total does kidded	383					

Table 7. Overall potential and realized kidding rate by breeding number.

Breeding Nº	Goats kidded	Open	Total Bred	PNL	Kidded + PNL	Kidding Rate (%)	
						Potential	Realized
1	246	207	533	80	326	61	46
2	103	128	272	41	144	53	38
3	27	33	67	7	34	51	40
4	7	0	7	0	7	100	100
Total	**383**	**96**	**879**	**128**	**511**	**58**	**44**

Table 8. Kidding rate to first fixed-time breeding (based on mated goats) by breeding procedure and progesterone time length protocol.

		Intrauterine (LAI)			Natural Service (pen)						Transcervical (TrAI)			
Progesterone (P4) protocol		L	N	S	L	L SynB	N	NNT	S	XL	L	N	S	Grand Total
Kidded	Nº	11	14	7	48	21	30	58	34	5	3	7	8	246
	%	33	56	14	52	72	61	68	50	20	12	32	27	46
Open	Nº	22	11	42	45	8	19	27	34	20	22	15	22	287
	%	67	44	86	48	28	39	32	50	80	88	68	73	54
Total Nº mated		33	25	49	93	29	49	85	68	25	25	22	30	533
Average kidding rate		30%			57%		66%				23%			
					56%									

Table 9. Kidding rate to first fixed-time breeding (based on fertile goats) by breeding procedure and progesterone time length protocol.

		Intrauterine (LAI)			Natural Service (pen)						Transcervical (TrAI)			
Progesterone (P4) Protocol		L	N	S	L	L SynB	N	NNT	S	XL	L	N	S	Grand Total
Kidded	Nº	11	14	7	48	21	30	58	34	5	3	7	8	246
	%	61	78	32	73	88	100	83	89	63	60	88	42	75
Open	Nº	7	4	15	18	3	0	12	4	3	2	1	11	287
	%	39	22	68	27	12	0	17	11	37	40	12	58	25
Total Nº fertile		18	18	22	66	24	30	70	38	8	5	8	19	326
Average kidding rate		55%			77%		88%				62%			
					83%									

Table 10. Overall fertile breedings and fertility according to breeding number.

	Kidded		Open		Prenatal loss[a]		Total breedings		Fertile	
Breeding Nº	Nº	%[d]	Nº	%	Nº	%	Nº	%	Nº	%
1[b,c]	246	46	207	39	80	15	533	100	326	61
2[e]	103	38	128	47	41	15	272	100	144	53
3[e]	27	40	33	49	7	10	67	100	34	51
4[e]	7	100	0	0	0	0	7	100	7	100
Total Nº	383	44	368	42	128	14	879	100	511	58

[a] Females with embryonic loss, terminated pregnancy and death of pregnant goats .

[b] Includes all three methods of breeding (NS, TrAI, LAI).

[c] Includes all methods of hormonal synchrony (S, L and XL) and goats not synchronized.

[d] Percentage calculated on the basis of total goats bred per breeding group.

[e] Breedings with no estrus synchronization and using only NSp (not fixed-time bred).

Table 11. Fertility to first fixed-time breeding by breeding procedure and progesterone time of exposure protocol.

		Intrauterine (LAI)			Natural Service (pen)						Transcervical (TrAI)			
Progesterone (P4) Protocol		L	N	S	L	L SynB	N	NNT	S	XL	L	N	S	Grand Total
Fertile	Nº	18	18	22	66	24	30	70	38	8	5	8	19	326
	%	55	72	45	71	83	61	82	56	32	20	36	63	61
Open	Nº	15	7	27	27	5	19	15	30	17	20	14	11	207
	%	45	28	55	29	17	39	18	44	68	80	64	37	39
Total Nº in breeding group		33	25	49	93	29	49	85	68	25	25	22	30	533
Average fertility rate		54%			74%		75%				42%			
					67%									

Table 12. Prolificacy and fecundity of first fixed-time breeding as a function of P4 exposure and breeding procedure.

	Intrauterine AI				Natural Service (pen)							Transcervical AI				Grand Total
Kids born alive	**L**	**N**	**S**	**Total**	**L**	**L-SynB**	**N**	**NNT**	**S**	**XL**	**Total**	**L**	**N**	**S**	**Total**	
1	3	3	1	7	14	0	11	18	13	3	59	2	5	2	9	75
2	14	14	8	36	44	28	32	68	28	2	202	2	2	8	12	250
3	0	12	3	15	24	15	9	3	18	0	69	0	0	3	3	87
4	0	0	0	0	12	4	0	4	0	4	24	0	0	4	4	28
Grand Total	17	29	12	58	94	47	52	93	59	9	354	4	7	17	28	440
Kidded	11	14	7	32	48	21	30	58	34	5	196	3	7	8	18	246
Prolificacy[a]	1.5	2.1	1.7	**1.8**	2.0	2.2	1.7	1.6	1.7	1.8	**1.8**	1.3	1.0	2.1	**1.6**	**1.8**
Goats mated	33	25	49	107	93	29	49	85	68	25	349	25	22	30	77	533
Fecundity[b]	0.51	1.16	0.24	**0.54**	1.01	1.62	1.06	1.09	0.87	0.36	**1.01**	0.16	0.32	0.57	**0.36**	**0.83**

[a]Calculated as total kids born alive/ females kidding.

[a]Calculated as total kids born alive/ females mated.

Table 13. Number of goats bred by breed and by breeding number.

	Breeding N°					
Breed	**1**	**2**	**3**	**4**	**Total Bred N°**	**%**
Alpine	92	39	10	---	141	16
Boer	109	49	9	2	169	19
Spanish	40	17	4	---	61	7
Stiff-Leg	27	24	16	3	70	8
X-Breds	213	108	23	2	346	39
Angora	52	35	5	---	92	11
Grand Total	533	272	67	7	879	100

Table 14. Prolificacy and fecundity by goat breed and breeding number.

	Litter Size						Kids	Total does:			
	0	1	2	3	4	5	Nº	Mated	Kidded	Prolif.	Fecund.
	Does	No of kids born									(%)
Breeding 1 ►	209	122	314	111	28	5	580	533	324	1.8	109
Alpine	31	20	52	36	8	5	121	92	61	2.0	132
Angora	27	17	16	--	--	--	33	52	25	1.3	63
Boer	45	16	76	33	8	--	133	109	67	2.0	122
Spanish	9	12	34	12	--	--	58	40	33	1.8	145
T. Stiff Leg	22	6	6		--	--	12	27	9	1.3	44
X-Bred	98	51	130	30	12	--	223	213	129	1.7	105
Breeding 2 ►	137	50	154	33	4	--	241	271	139	**1.7**	**89**
Alpine	31	10	28	3	--	--	41	39	25	1.6	105
Angora	27	15	2		--	--	17	35	16	1.1	49
Boer	42	5	36	9	--	--	50	48	26	1.9	102
Spanish	7	5	18	--	--	--	23	17	14	1.6	135
T. Stiff Leg	18	3	6	--	--	--	9	24	6	1.5	38
X-Bred	56	12	64	21	4	--	101	108	52	1.9	94
Breeding 3 ►	33	16	32	6	--	--	54	67	34	**1.6**	**81**
Alpine	5	4	2	--	--	--	6	10	5	1.2	60
Angora	1	4	--	--	--	--	4	5	4	1.0	80
Boer	6	1	4	--	--	--	5	9	3	1.7	56
Spanish	1	--	6	--	--	--	6	4	3	2.0	150
T. Stiff Leg	10	2	8	--	--	--	10	16	6	1.7	63
X-Bred	10	5	12	6	--	--	23	23	13	1.8	100
Breeding 4 ►	--	2	10	--	--	--	12	7	7	**1.7**	**171**
Boer	--	1	2	--	--	--	3	2	2	1.5	150
T. Stiff Leg	--	1	4	--	--	--	5	3	3	1.7	167
X-Bred	--	--	4	--	--	--	4	2	2	2.0	200
Total	**375**	**190**	**510**	**150**	**32**	**5**	**887**	**878**	**504**	**1.8**	**101**

Table 15. Prolificacy means comparisons for each sequential breeding (for each pair using Student's t).

Level	(P>0.541)	Mean
1	A	1.7886
2	A	1.7282
3	A	1.7037
4	A	1.4286

Table 16. Prolificacy means comparison for each phenotype (for each pair using Student's t).

Level	(P<0.0009)	Mean
Boer	A	1.899
Spanish	A	1.897
Alpine	A	1.781
X-Breds	A	1.761
Stiff-Legs	A	1.750
Angora	B	1.235

Table 17. Fecundity means comparison for each breeding (for each pair using Student's t).

Level	(P< 0.0207)	Mean
4	A	1.4286
1	A	0.8256
3	A B	0.6866
2	B	0.6544

Table 18. Fecundity means comparison for each breed (for each pair using Student's t).

Level	(P<0.0172)	Mean
Spanish	A	1.2131
Boer	B	0.8876
Alpine	B	0.8085
X-Breds	B	0.7890
Angora	C	0.4565
Stiff-Leg	C	0.3000

Table 19. Odds ratios (OR) for given mean comparison and associated probabilities (P) for length of progesterone exposure using natural service (pen) breeding procedure.

	Natural Service (pen)																	
	Conception Rate			Pregnancy Rate			Fertility			Prolificacy			Fecundity			Kidding Rate (fertile doe basis)		
Comparison	%	OR	P	%	OR	P	%	OR	P	%	OR	P	%	OR	P	%	OR	P
LngSyn – Lng	83-71	2.0	**>0.207**	83-70	2.1	**>0.190**	72-52	2.5	**<0.049**	2.2-2.0	1.1	**>0.464**	162-101	36	**<0.000**	88-73	2.6	**>0.142**
Lng – None	71-61	1.5	**>0.238**	70-62	1.4	**>0.432**	52-61	1.5	**>0.273**	2.0-1.7	1.2	**>0.449**	101-106	5.4	**<0.000**	73-100	>15	**<0.006**
NNT – Lng	82-71	1.9	**>0.073**	86-70	2.6	**<0.042**	68-52	2.0	**<0.024**	1.6-2.0	1.3	**>0.117**	109-101	8.1	**<0.000**	83-73	1.8	**>0.154**
Lng – Short	71-56	1.9	**<0.048**	70-57	1.7	**>0.148**	52-50	1.1	>0.839	2.0-1.7	1.2	**>0.433**	101-87	14.3	**<0.000**	73-89	3.2	**=0.044**
Lng – XL	71-32	5.2	**<0.001**	70-20	9.2	**<0.000**	52-20	4.3	**<0.005**	2.0-1.8	1.1	**>0.803**	101-36	167	**<0.000**	73-63	1.6	**>0.544**
LngSyn – None	83-61	3.0	**<0.047**	83-62	2.9	**<0.050**	72-61	1.7	**>0.315**	2.2-1.7	1.3	**>0.189**	162-106	6.8	**<0.000**	88-100	>100	**<0.05**
Lng-Syn – NNT	83-82	1.0	**>0.960**	83-86	1.3	**>0.720**	72-68	1.2	**>0.673**	2.2-1.6	1.5	**<0.034**	162-109	4.5	**<0.000**	88-83	1.4	**>0.592**
Lng-Syn –Shrt	83-56	3.8	**<0.012**	83-57	3.6	**<0.016**	72-50	2.6	**<0.041**	2.2-1.7	1.3	**>0.175**	162-87	2.5	**<0.000**	88-89	1.2	**>0.811**
Lng-Syn – XL	83-32	10.2	**<0.001**	83-20	19.2	**<0.000**	72-20	10.5	**<0.001**	2.2-1.8	1.2	**>0.576**	162-36	4.6	**<0.000**	88-63	4.2	**>0.116**
NNT – None	82-61	3.0	**<0.007**	86-62	3.6	**<0.007**	68-61	1.4	**>0.410**	1.6-1.7	1.1	**>0.576**	109-106	1.5	**>0.538**	83-100	>100	**>0.071**
None – Short	61-56	1.2	**>0.563**	62-57	1.2	**>0.578**	61-50	1.6	>0.228	1.7-1.7	1.0	**>0.994**	106-87	2.64	**>0.000**	100-89	>100	**<0.050**
None – XL	61-32	3.4	**<0.018**	62-20	6.3	**<0.002**	61-20	6.3	**<0.001**	1.7-1.8	1.1	**>0.903**	106-36	30.8	**<0.000**	100-63	>100	**<0.001**
NNT – Short	82-56	3.7	**<0.001**	86-57	4.5	**<0.001**	68-50	2.1	**<0.023**	1.6-1.7	1.1	**>0.556**	109-87	1.7	**<0.000**	83-89	1.8	**>0.355**
NNT – XL	82-32	9.9	**<0.000**	86-20	24	**<0.000**	68-20	8.6	**<0.000**	1.6-1.8	1.2	**>0.673**	109-36	20.7	**<0.000**	83-63	2.9	**>0.166**
Short – XL	56-32	2.7	**<0.042**	57-20	5.3	**<0.002**	50-20	4.0	**<0.010**	1.7-1.8	1.0	**>0.905**	87-36	11.7	**<0.000**	89-63	5.1	**>0.053**

Table 20. Odds ratios (OR) for given mean comparison and associated probabilities (P) for length of progesterone protocol using laparoscopically-aided intrauterine AI (LAI) breeding procedure.

	Laparoscopic AI																	
	Conception Rate			Pregnancy rate			Fertility			Prolificacy			Fecundity			Kidding Rate		
Comparison	%	OR	P	%	OR	P	%	OR	P	%	OR	P	%	OR	P	%	OR	P
None – Long	72-55	2.1	>0.174	71-61	1.6	>0.424	56-33	2.5	>0.084	2.1-1.5	1.5	>0.293	116-52	6.8	**<0.000**	78-61	2.2	>0.277
None – Short	72-45	1.5	>0.391	71-24	7.5	**<0.000**	56-14	1.6	>0.426	2.1-1.7	1.2	>0.571	116-24	22.3	**<0.000**	78-32	3.4	>0.063
Long – Short	55-45	3.2	**<0.027**	61-24	4.7	**<0.001**	33-14	2.4	>0.107	1.5-1.7	1.2	>0.724	52-24	3.3	**<0.012**	61-32	7.5	**<0.004**

Table 21. Odds ratios (OR) for given mean comparison and associated probabilities (P) for length of progesterone exposure using transcervical AI breeding procedure.

	Transcervical AI																	
	Conception Rate			Pregnancy Rate			Fertility			Prolificacy			Fecundity			Kidding Rate		
Comparison	%	OR	P	%	OR	P	%	OR	P	%	OR	P	%	OR	P	%	OR	P
None – Long	36-20	2.3	>0.210	27-26	1.1	>0.928	32-12	3.4	>0.097	1.0-1.3	17.5	>0.189	32-16	2.5	>0.201	88-60	4.7	>0.252
None – Short	36-63	6.9	**<0.002**	27-26	1.1	>0.945	32-27	2.7	>0.175	1.0-2.1	37.1	**<0.030**	32-57	2.8	>0.075	88-42	9.6	**<0.031**
Long – Short	20-63	3	**>0.055**	26-26	1	>0.986	12-27	1.3	>0.685	1.3-2.1	2.1	>0.374	16-57	6.9	**<0.003**	60-42	2.1	>0.474

Table 22. Sample size by PG600 dose level and breeding procedure.

Gonadotropin dose level			Breeding procedure				
PG600 (mL)	**eCG (IU)**	**hCG (IU)**	**LAI**	**NSp**	**TrAI**	**Total**	**(%)**
5.0	400	200	30	173	28	231	43
1.75	140	70	49	113	34	196	37
0.0	---	---	28	63	15	106	20
		Grand total	107	349	77	533	100

Table 23. First service conception rate (adjusted for fertile females based on prenatal loss) by breeding procedure and PG600 dose.

1st breeding	LAI	NSp	TrAI	Total		
PG600 (0.0 mL)				**231**		
Open	11	52	16	79		
Pregnant	15	94	6	115		
SB	---	11	2	13		
Aborted	1	3	1	5		
EM	3	13	3	19		
PG600 (1.75 mL)				**196**		
Open	22	43	23	88		
Pregnant	10	49	5	64		
SB	2	5	---	7		
Aborted	3	3	---	6		
EM	12	12	6	30		
MD	---	1	---	1		
PG600 (5.0 mL)				**106**		
Open	17	19	6	42		
Pregnant	4	35	5	44		
SB	1	2	---	3		
Aborted	---	1	---	1		
EM	5	5	4	14		
C	1	---	---	1		
MD	---	1	---	1		
Grand Total	107	349	77	**533**		
Total open	50	114	45	209 (39%)		
Total pregnant	29	108	16	223 (42%)		
Total PPNL	28	57	16	101 (19%)		
Total conception	57	165	32	324 (61%)		

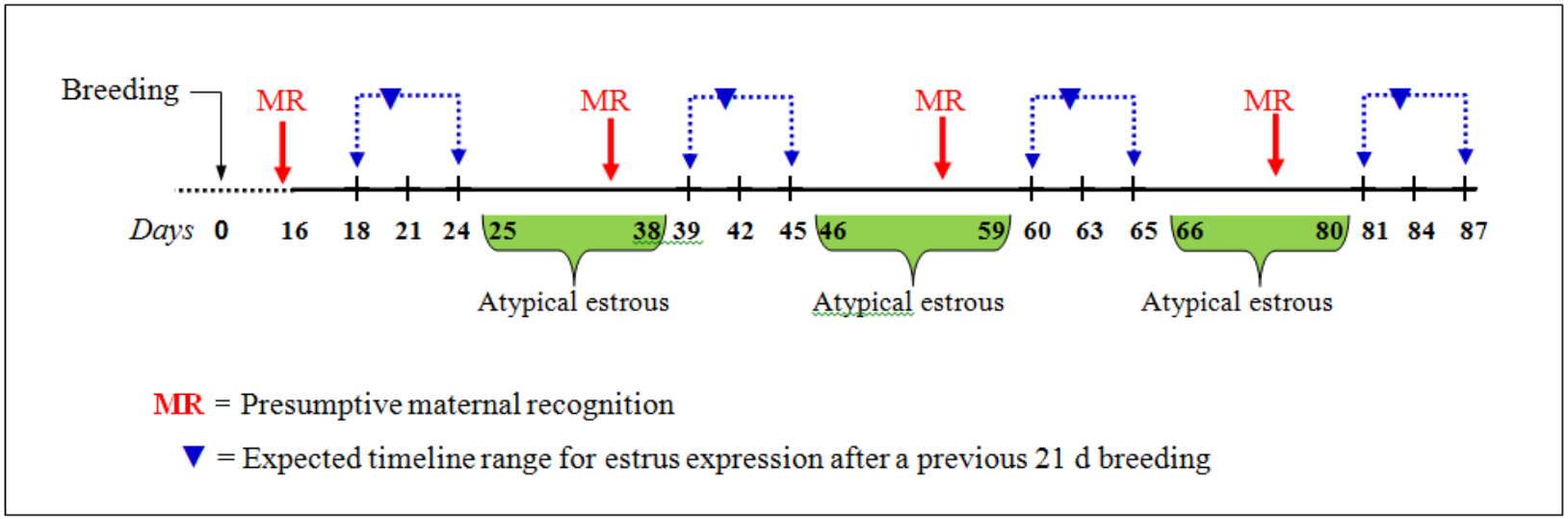

Breeding
MR
MR
MR
MR
Days 0 16 18 21 24 25 38 39 42 45 46 59 60 63 65 66 80 81 84 87
Atypical estrous
Atypical estrous
Atypical estrous
MR = Presumptive maternal recognition
▼ = Expected timeline range for estrus expression after a previous 21 d breeding

Figure 1. Atypical estrous intervals used to describe estimated conception in bred goats. It was considered that conception (i.e., embryo attachment) had occurred if at least one embryo attached to the uterus under the premise that there was no return to estrus by day 17 up to day 25 PB or if there was a return to estrus, after having been bred, in an atypical estrous cycle period of days (*i.e.,* > 24 to <39 for first cycle post breeding and > 24 to <39, > 45 to < 60 d and >66 < 81 for second cycle post breeding).

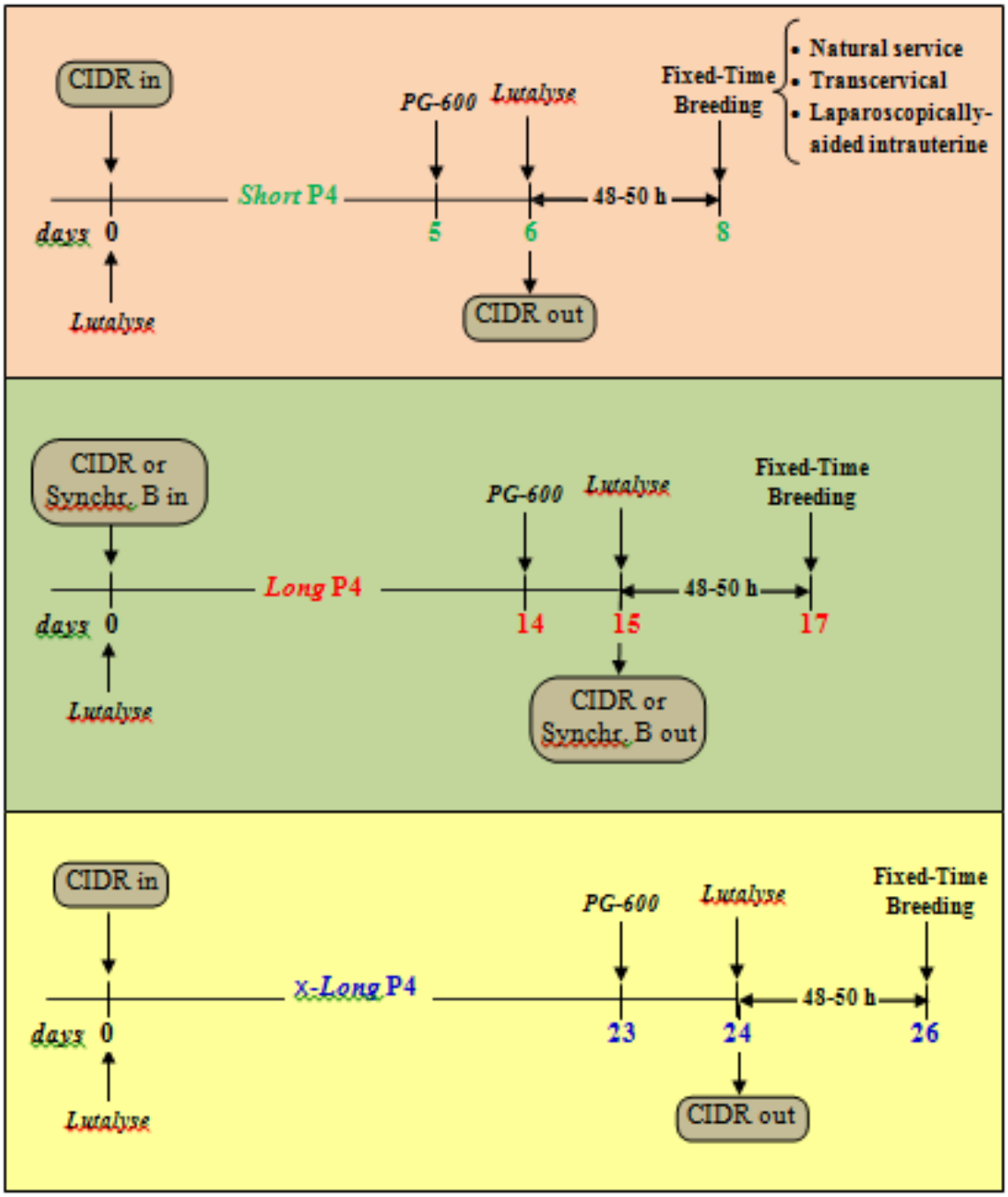
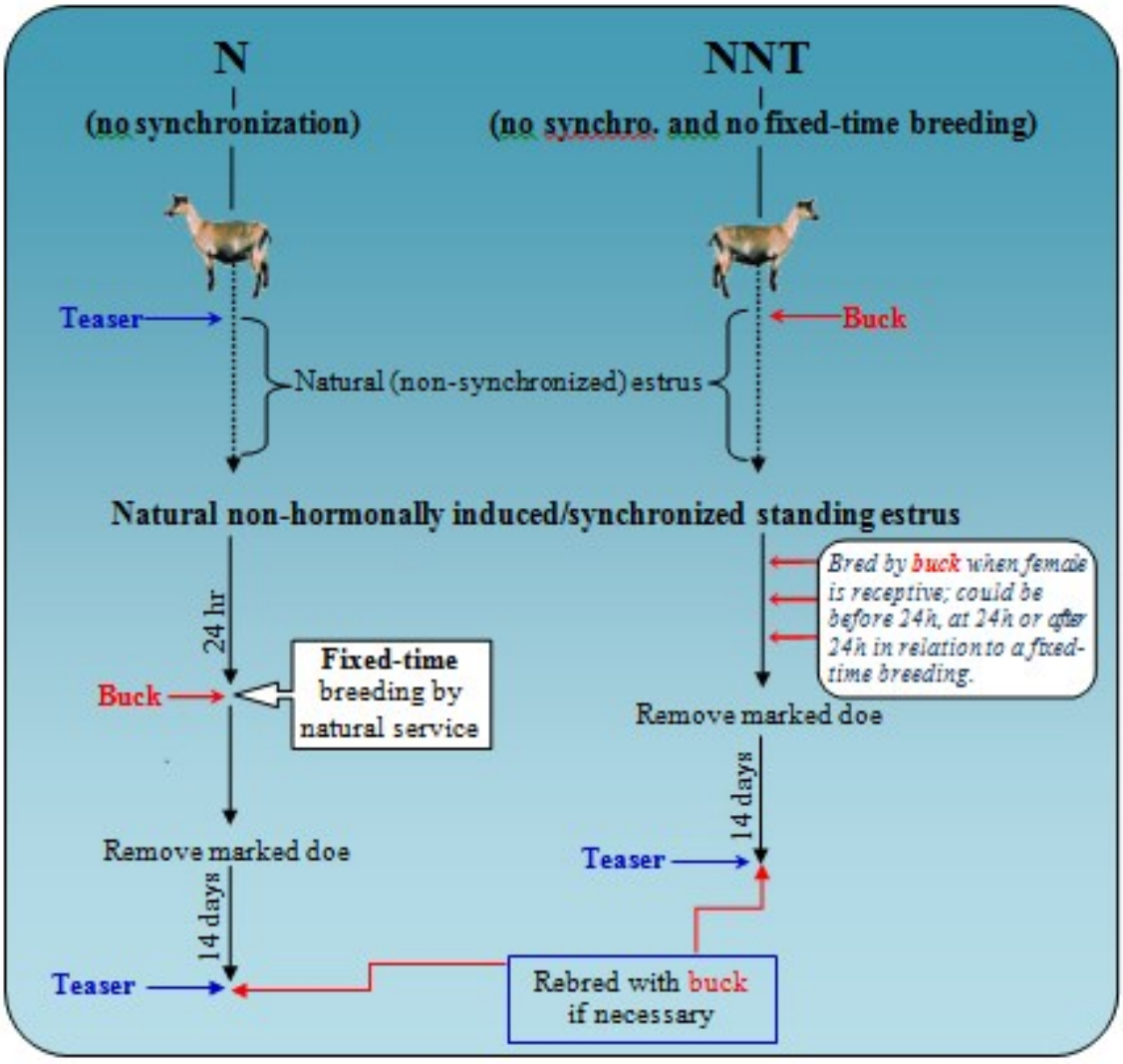

CIDR in
PG-600
Lutalyse
Fixed-Time Breeding
• Natural service
• Transcervical
• Laparoscopically-aided intrauterine
Short P4
48-50 h
days 0
5
6
8
Lutalyse
CIDR out
CIDR or Synchr. B in
PG-600
Lutalyse
Fixed-Time Breeding
Long P4
48-50 h
days 0
14
15
17
Lutalyse
CIDR or Synchr. B out
CIDR in
PG-600
Lutalyse
Fixed-Time Breeding
x-Long P4
48-50 h
days 0
23
24
26
Lutalyse
CIDR out

Figure 2. Estrus/ovulation synchronization protocols. CIDR and prostaglandin (Lutalyse) was given on day zero. First panel shows short P4 protocol exposure of 5-6 d. The second panel shows long P4 protocol exposure of 14-15 d where P4 was provided as CIDR and, in a different independent group, P4 was also given in the form of a subcutaneous ear implant (Sincro-Mate-B; SMB). The third panel shows the x-long P4 protocol exposure of 24d. Chorionic gonadotropin (eCG and hCG; PG600) was given 24 h before removal of CIDR or SMB. A second dose of Lutalyse was given at the time of P4 removal. In all four treatment groups breeding was accomplished 48 to 50 h after P4 removal. As shown in the first panel all 4 treatments were assigned to one of three breeding procedures: Natural service (NS), transcervical artificial insemination (TrAI), or laparoscopically-aided intrauterine AI (LAI).


N
(no synchronization)
NNT
(no synchro. and no fixed-time breeding)
Teaser
Buck
Natural (non-synchronized) estrus
Natural non-hormonally induced/synchronized standing estrus
24 hr
Buck
Fixed-time breeding by natural service
Bred by buck when female is receptive; could be before 24h, at 24h or after 24h in relation to a fixed-time breeding.
Remove marked doe
14 days
Teaser
Remove marked doe
14 days
Teaser
Rebred with buck if necessary

.

Figure 3. Breeding protocol for non-synchronized control groups. Fixed-time (left tract; N) and non-fixed-time goats bred by natural service (NNT). The influence of fixed-time breeding on RP was determined using a control group of non-estrus synchronized goats (N) monitored for standing estrus using epididymectomized bucks (teasers) fitted with marking breeding harness. Twenty four h after a female was reported in standing estrus or was observed to have been marked it was taken to the buck for breeding. Bucks were allowed to breed females in estrus only once and does were removed from the pen immediately after the first breeding. A second cohort of control goats (NNT) was placed with bucks and allowed to breed when both male and female were receptive. In these latter cohort, Bucks were also allowed to breed females in estrus only once and does were removed from the pen immediately after the first breeding. In both the N and NNT groups does were placed with a teaser 14 d after the first breeding and re-bred with a buck if necessary.

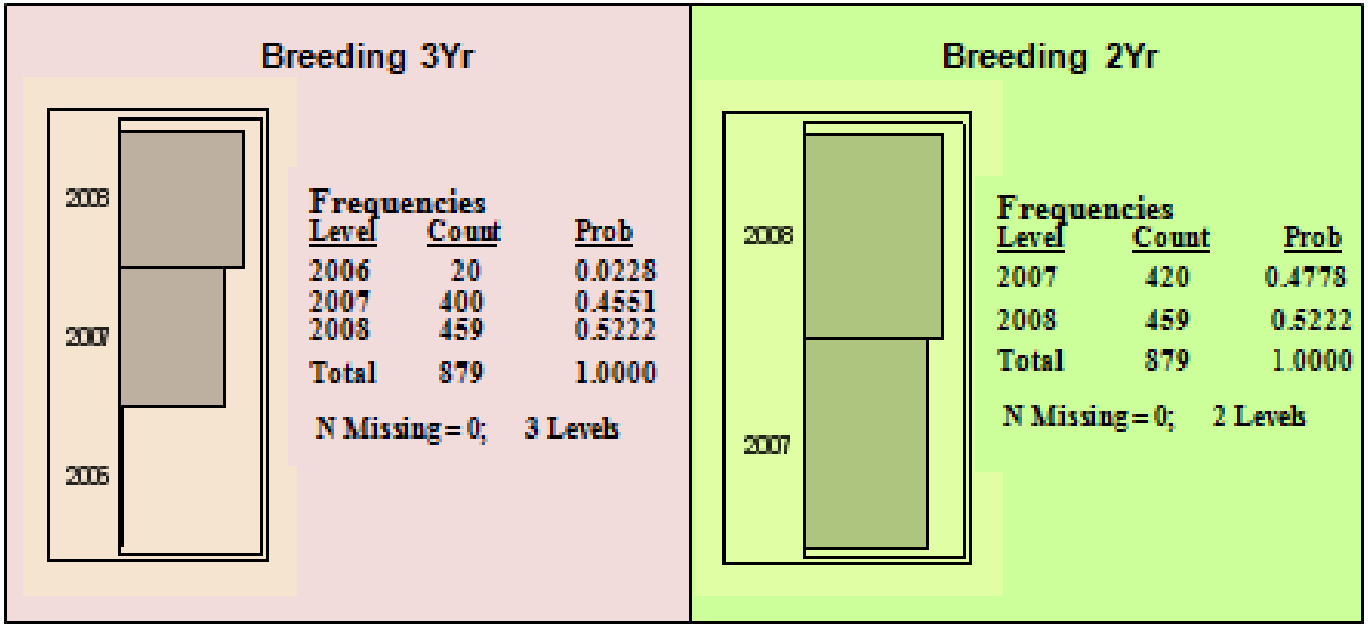

Breeding 3Yr
2008
2007
2006
Frequencies
Level Count Prob
2006 20 0.0228
2007 400 0.4551
2008 459 0.5222
Total 879 1.0000
N Missing = 0; 3 Levels
Breeding 2Yr
2008
2007
Frequencies
Level Count Prob
2007 420 0.4778
2008 459 0.5222
Total 879 1.0000
N Missing = 0; 2 Levels

Figure 4. Goat distribution by 3 y (’08 – ’07 – ’06) and by 2 y (’08 – ’07). Grouping data shown in left panel, included two breeding years (2007 and 2008) and a partial breeding season in 2006. The data from 2006 contributed 2.3% to the total observations and was judged to be insufficient (n=20) to validly use it as a categorizing variable. Therefore, all 2006 data was re-coded as “year 2007” as shown in the right panel.

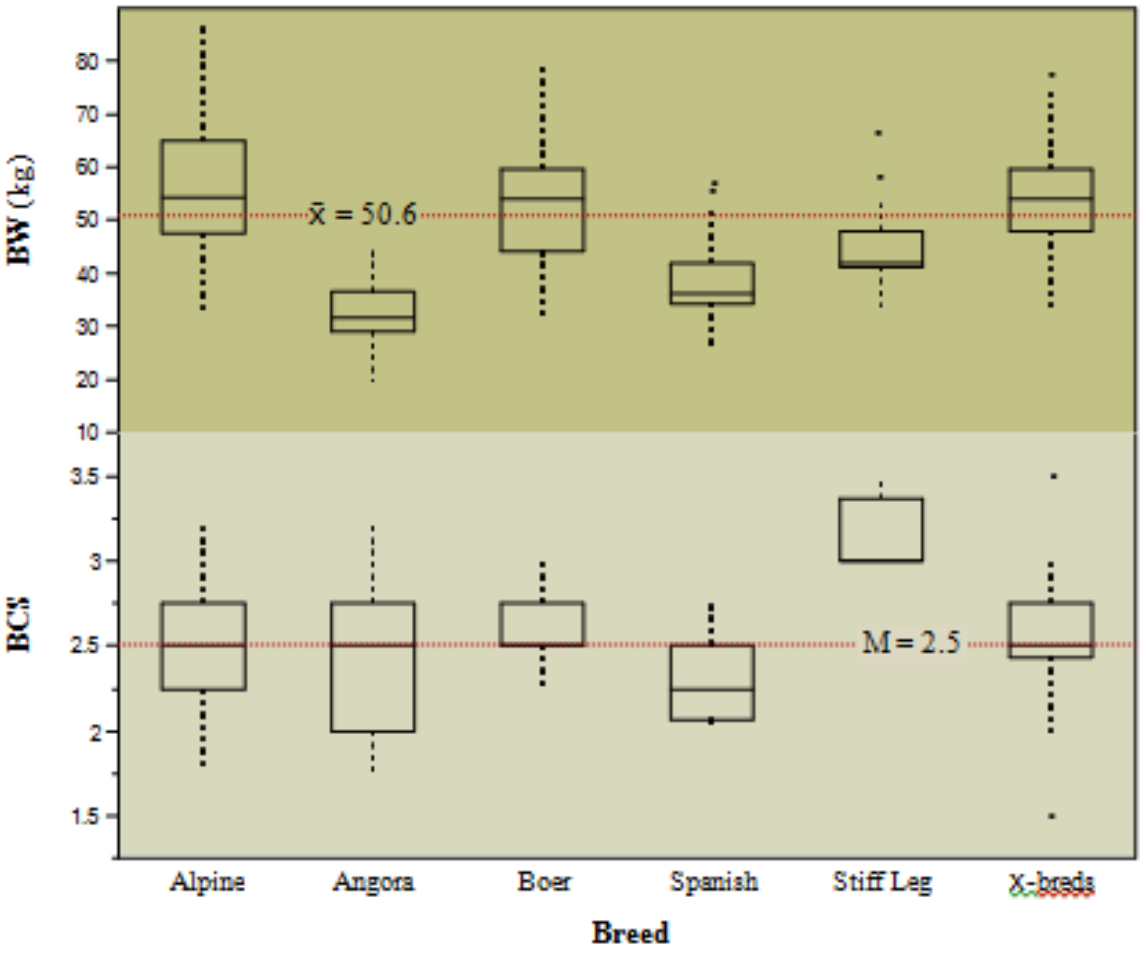

BW (kg)
80
70
60
50
40
30
20
10
x̄ = 50.6
BCS
3.5
3
2.5
2
1.5
M = 2.5
Alpine
Angora
Boer
Spanish
Stiff Leg
X-breds
Breed

Figure 5. Overall average body weight (kg) and modal body condition score (BCS) at time of breeding. Depending on breed the overall mean and ±SD of body weight was 57.9 ± 9.8 kg and the most common body BCS on a scale of 1 to 5 was 2.75.

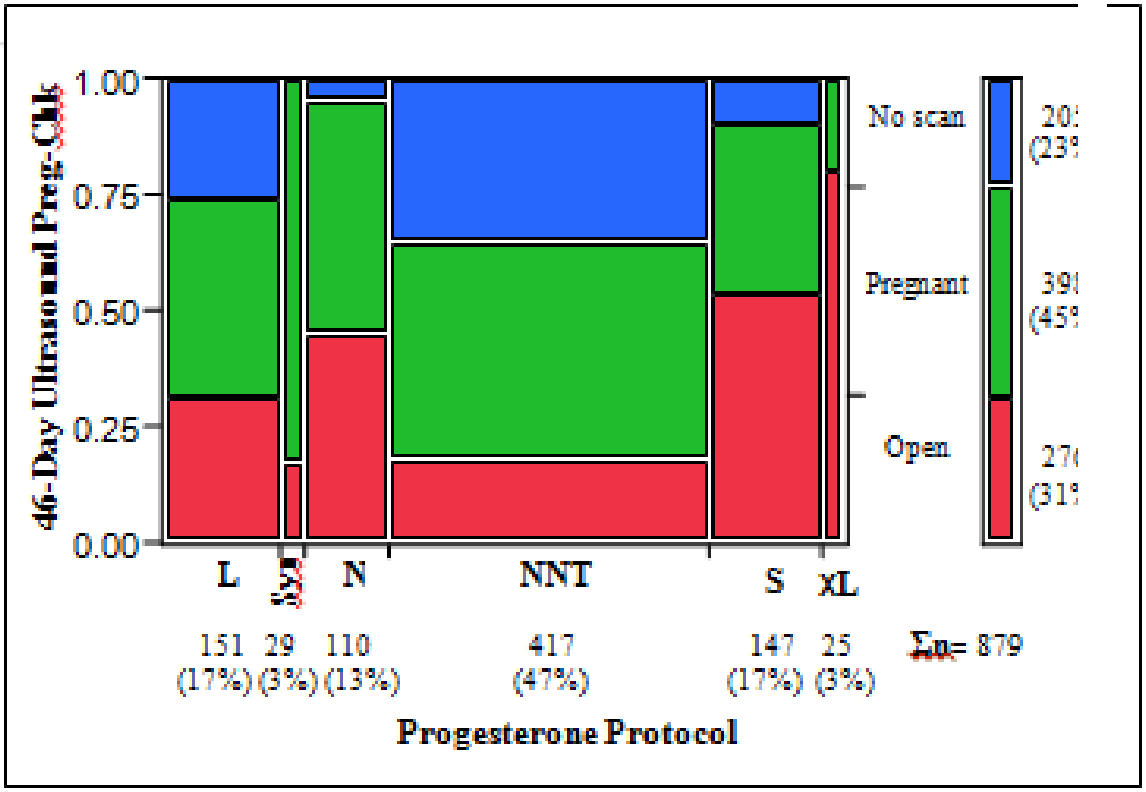

46-Day Ultrasound Preg-Chk
1.00
0.75
0.50
0.25
0.00
No scan
Pregnant
Open
L
N
NNT
S
XL
151 (17%)
29 (3%)
110 (13%)
417 (47%)
147 (17%)
25 (3%)
Σn= 879
Progesterone Protocol

Figure 6. Mosaic plot of goats checked by ultrasound imaging for reproductive status at 46-day post- breeding as a function of progesterone time exposure treatment. Long 12-14 d (L), Long 12-14 d with SMB (Syn), no synchronization (N), no synchronization and no fixed-time breeding (NNT), short 5-6 d (S), and extra-long 24 d (xL) Shown are the number of goats determined to be pregnant, not-pregnant, and goats that were not scanned.

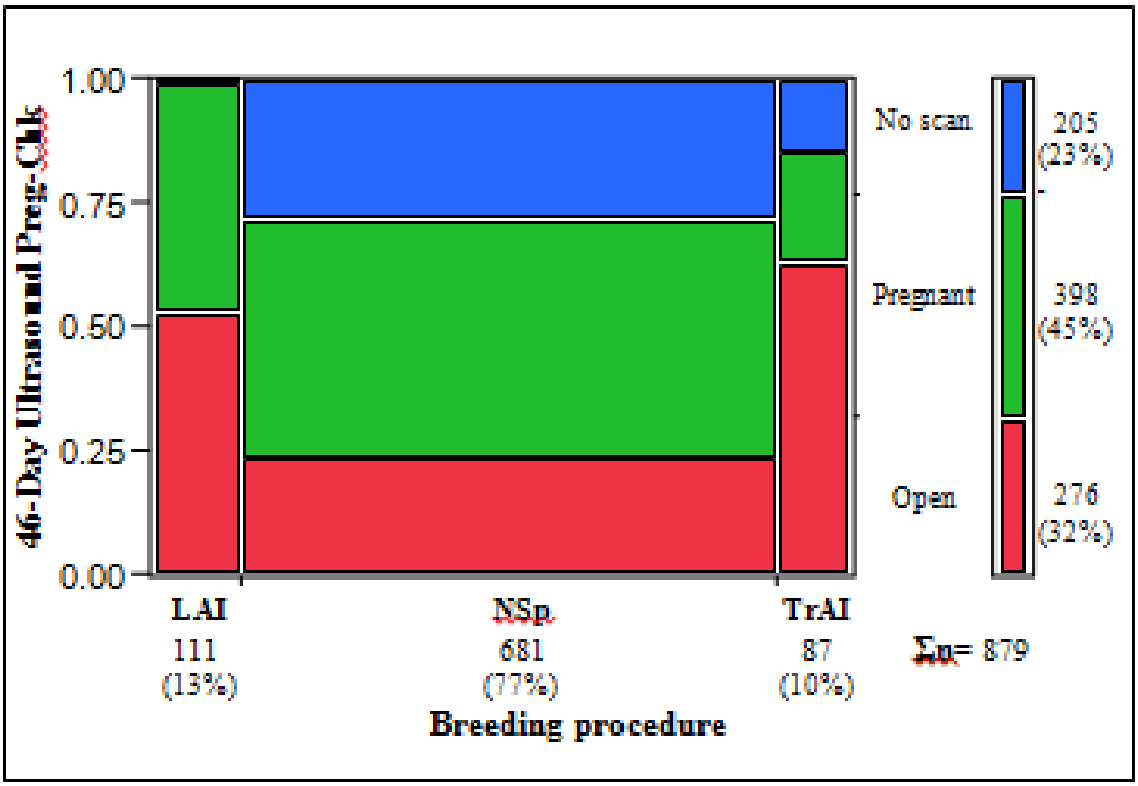

46-Day Ultrasound Preg-Chk
1.00
0.75
0.50
0.25
0.00
LAI
111
(13%)
NSp
681
(77%)
TrAI
87
(10%)
Breeding procedure
No scan
Pregnant
Open
205
(23%)
398
(45%)
276
(32%)
Σn= 879

Figure 7. Mosaic plot of goats checked by ultrasound imaging for reproductive status at 46-day post- breeding as a function of breeding procedure treatment. Natural service (NS), transcervical artificial insemination (TrAI), and laparoscopically-aided intrauterine AI (LAI) Shown are the number of goats determined to be pregnant, not-pregnant, and goats that were not scanned.

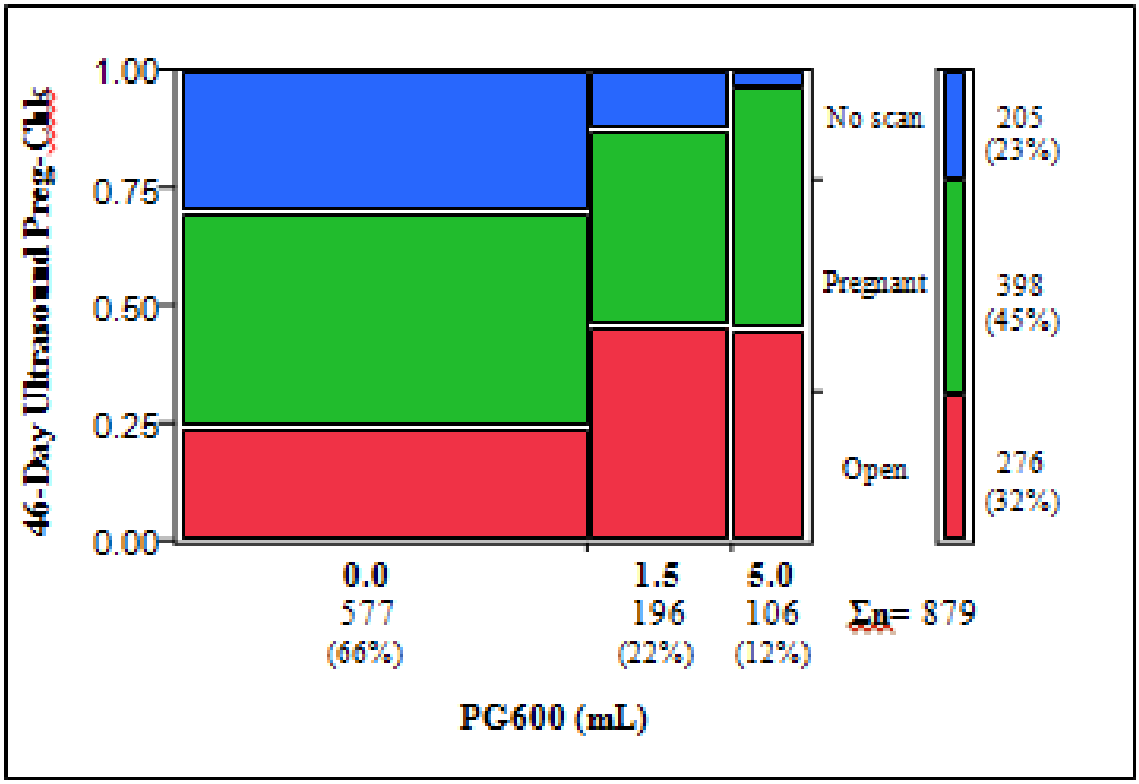

46-Day Ultrasound Preg-Chk
1.00
0.75
0.50
0.25
0.00
No scan
Pregnant
Open
205
(23%)
398
(45%)
276
(32%)
0.0
577
(66%)
1.5
196
(22%)
5.0
106
(12%)
Σn= 879
PG600 (mL)

Figure 8. Mosaic plot of goats checked by ultrasound imaging for reproductive status at 46-day post- breeding as a function of PG600 dose level treatment (none, 1.75 mL, and 5 mL). Shown are the number of goats determined to be pregnant, not-pregnant, and goats that were not scanned.

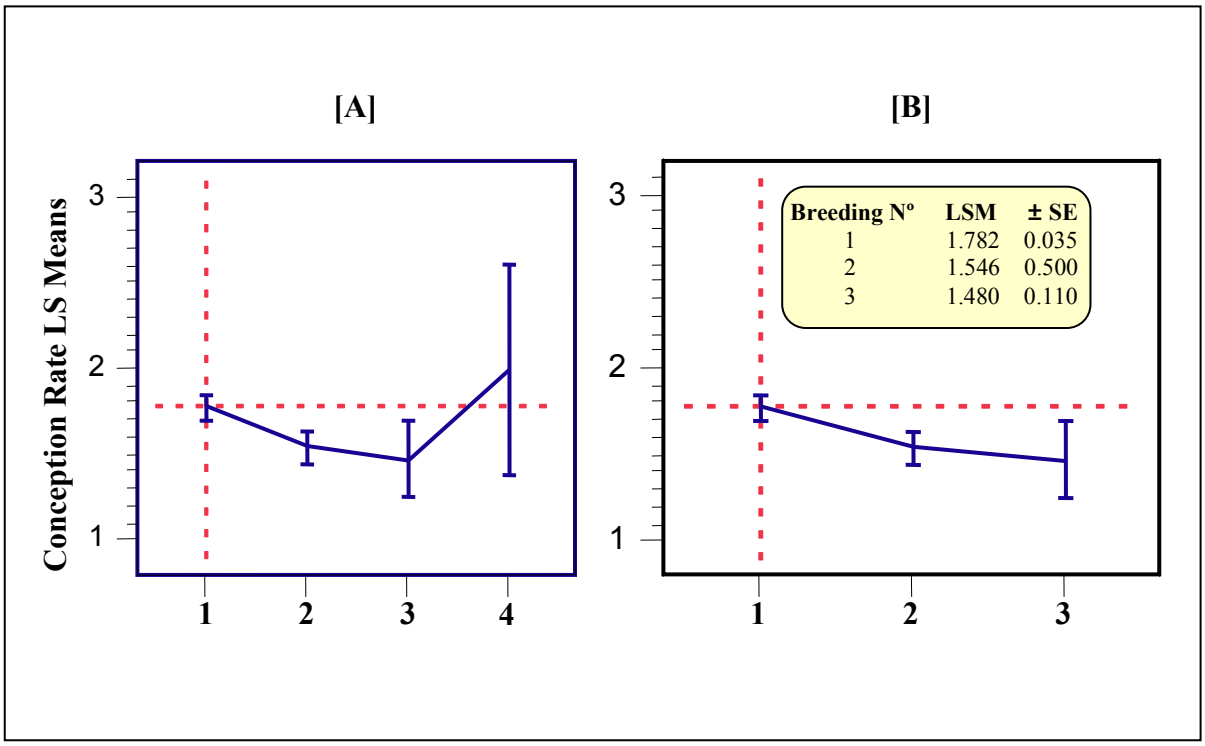

[A]
[B]
Conception Rate LS Means
3
2
1
1
2
3
4
Breeding Nº LSM ± SE
1 1.782 0.035
2 1.546 0.500
3 1.480 0.110

Figure 9. Least squares means (LSM) of **c**onception rate of goats as a function of breeding number.. This increasing variance effect, present from the first to the 4th breeding, can be clearly observed on panel [A]. Panel [B] shows the reduction in variability to a non-significant level when group 4 is not included in the evaluation of variance homogeneity.

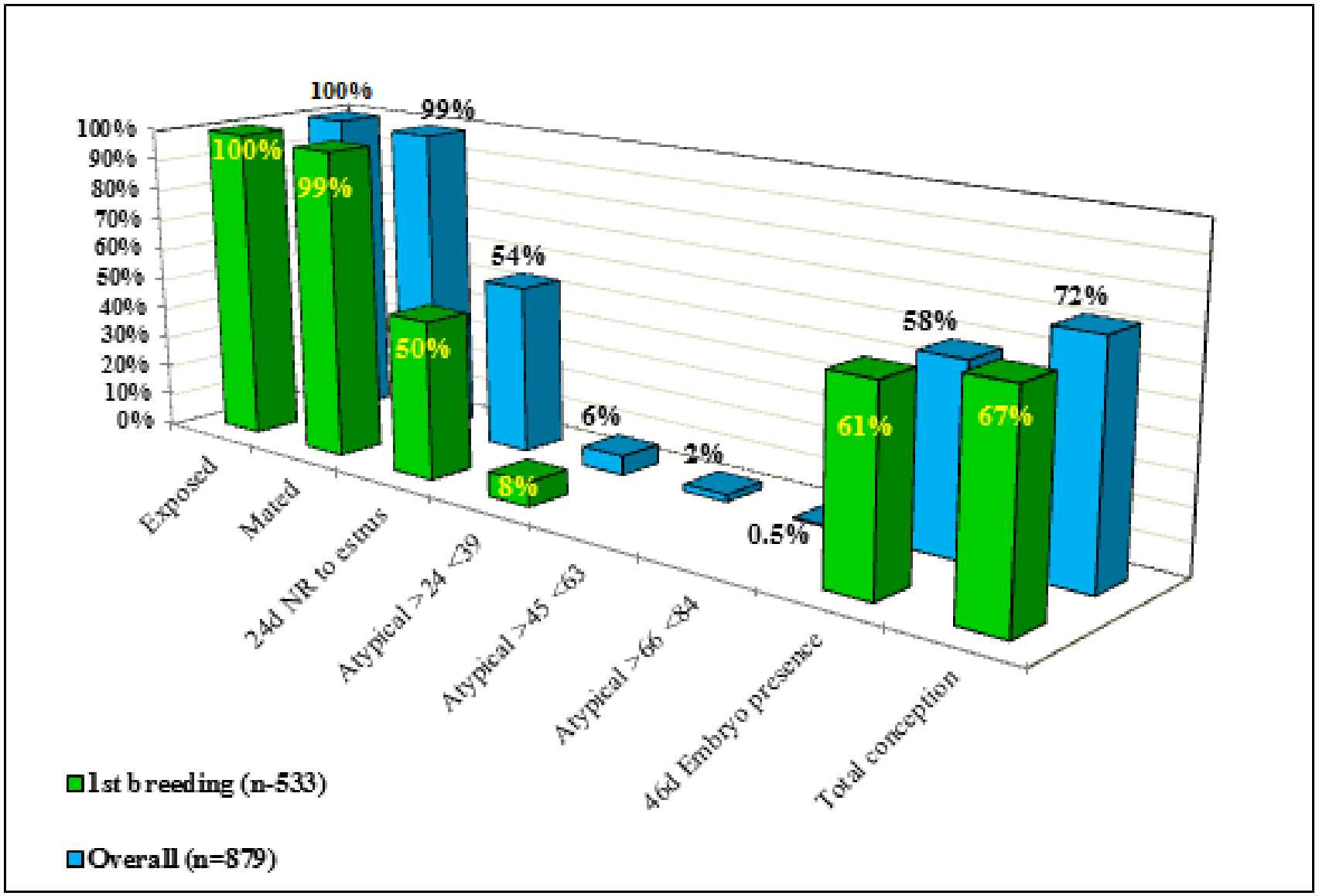
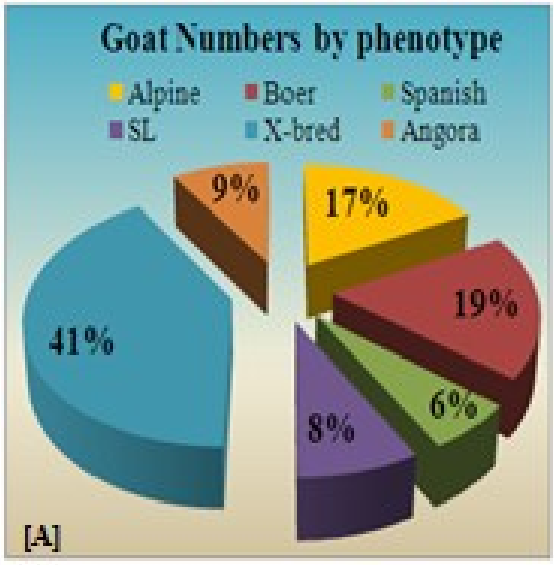

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100%
99%
54%
6%
2%
0.5%
58%
72%
100%
99%
50%
8%
61%
67%
Exposed
Mated
24d NR to estrus
Atypical >24 <39
Atypical >45 <63
Atypical >66 <84
46d Embryo presence
Total conception
1st breeding (n-533)
Overall (n=879)

Figure 10. Overall (blue) and first service (green) maternal conception rate. The number of mated or bred goats is very similar to the number exposed because of fixed-time breeding. Final CR is evaluated with all the direct and indirect information available (*i.e.,* 25 d non-return rates, presence of embryo(s) at 46 dPB ultrasound scan, and atypical estrous cycles lengths).The overall CR was estimated to be 72% (n= 879) and the first breeding 67% (n= 533). In each case estimated CR was improved by approximately 8% percent units by using atypical estrous expression.


Goat Numbers by phenotype
Alpine
Boer
Spanish
SL
X-bred
Angora
9%
17%
19%
41%
6%
8%
[A]

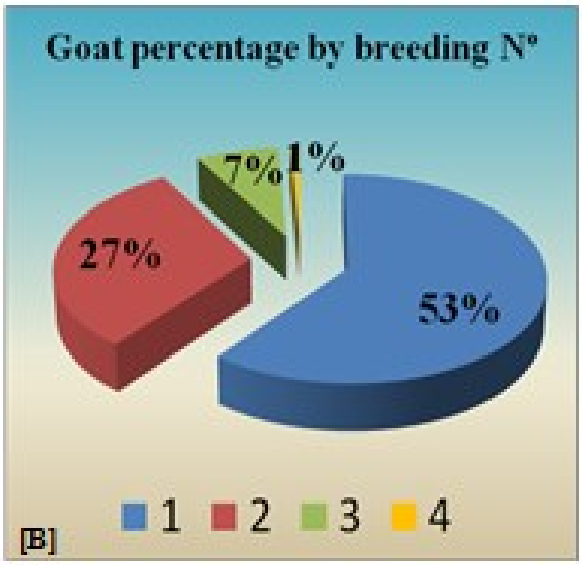

Goat percentage by breeding Nº
7%
1%
27%
53%
1
2
3
4
[B]

Figure 11. Goat grouping (%) by phenotype [A] and by breeding number [B]. Percentages are calculate on the basis of 879 bred goats and 126 goats bred with no semen delivery (TrAI-pass) (n= 1005).

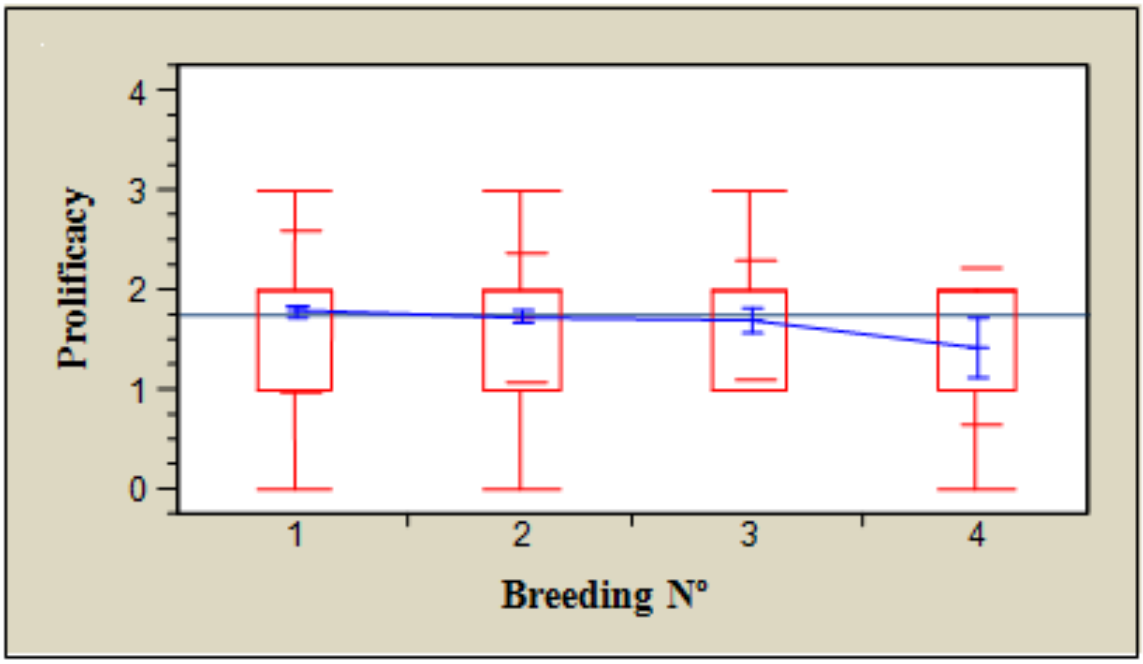

Prolificacy
4
3
2
1
0
1
2
3
4
Breeding N°

Figure 12. Box plot of prolificacy by breeding number. A tendency for a lower Pr to be associated with the number of times a goat was bred is noticeable with a drop in Pr particularly noticeable in the last breeding. Each box plot depicts a compact view of a variable's distribution, with quartiles and outliers, graphically depicting groups of numerical data through their five-number summaries: sample maximum, upper quartile, median, lower quartile, and sample minimum.

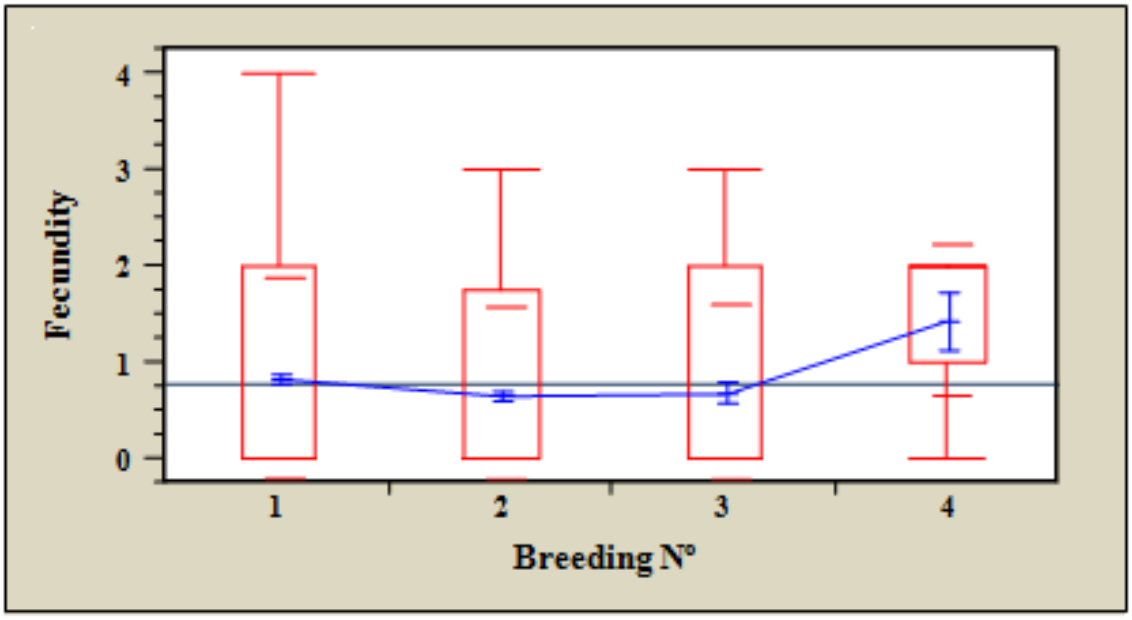

Fecundity
4
3
2
1
0
1
2
3
4
Breeding N°

Figure 13. Box plot of goat fecundity by breeding number. Fe is very stable among breeding 1 through 3with small variability. Breeding 4 has a greater Fc than the previous breeding but it also has more variability. Each box plot depicts a compact view of a variable's distribution, with quartiles and outliers, graphically depicting groups of numerical data through their five-number summaries: sample maximum, upper quartile, median, lower quartile, and sample minimum.

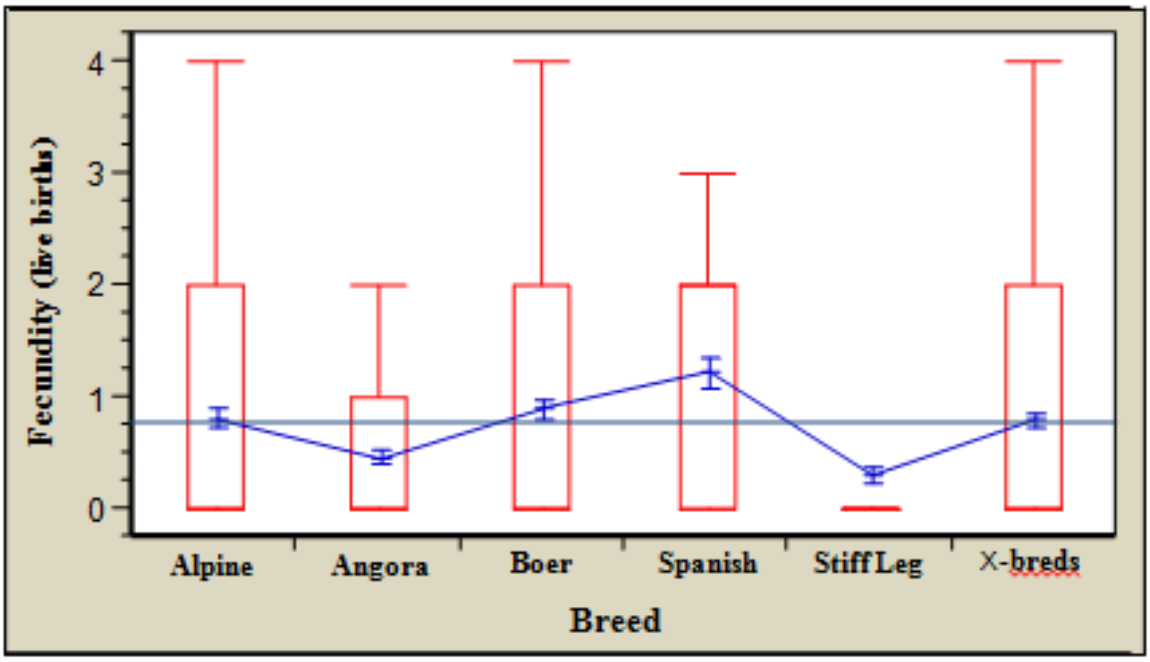

Fecundity (live births)
4
3
2
1
0
Alpine
Angora
Boer
Spanish
Stiff Leg
X-breds
Breed

Figure 14. Fecundity according to goat breed. The Spanish breed had more the 3× the level of Fc than the average Fc of the two lowest breeds. The Boer, Alpine and x-breds had similar Fc while expressing almost twice as much Fc than the Angora and Tennessee Stiff Leg breeds. These latter two breeds had similar Fc among themselves. Each box plot depicts a compact view of a variable's distribution, with quartiles and outliers, graphically depicting groups of numerical data through their five-number summaries: sample maximum, upper quartile, median, lower quartile, and sample minimum.

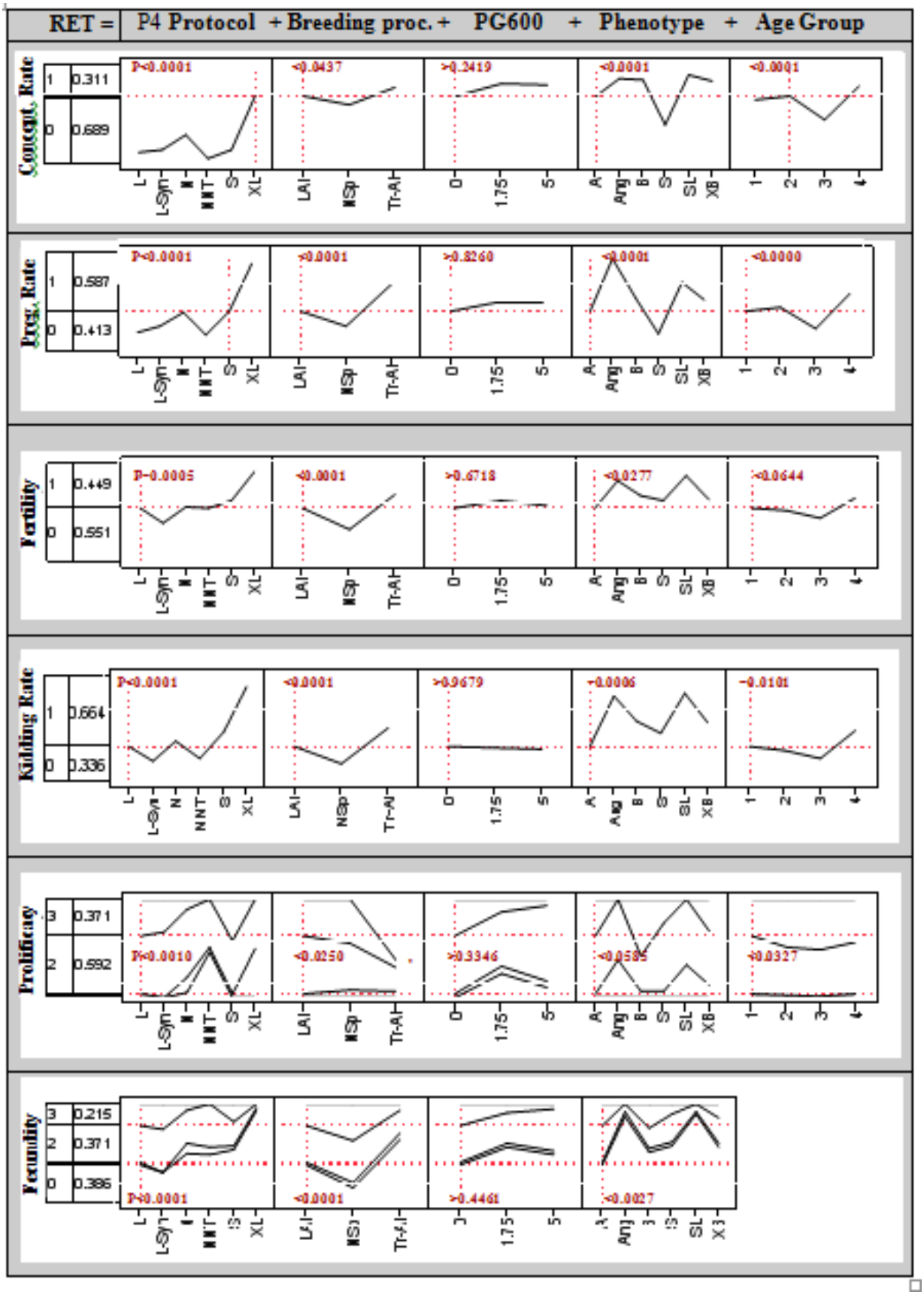

RET = P4 Protocol + Breeding proc. + PG600 + Phenotype + Age Group
Concept. Rate
1 0.311
0 0.689
P<0.0001
>0.0437
>0.2419
<0.0001
<0.0001
Preg. Rate
1 0.587
0 0.413
P<0.0001
<0.0001
>0.8260
<0.0001
<0.0000
Fertility
1 0.449
0 0.551
P=0.0005
<0.0001
>0.6718
<0.0277
<0.0644
Kidding Rate
1 0.664
0 0.336
P<0.0001
<0.0001
>0.9679
<0.0006
<0.0101
Prolificacy
3 0.371
2 0.592
P<0.0010
<0.0250
>0.3346
<0.0585
<0.0327
Fecundity
3 0.215
2 0.371
0 0.386
P<0.0001
<0.0001
>0.4461
<0.0027
L
L-Syn
N
NNT
S
XL
LAI
NSp
Tr-AI
0
1.75
5
A
Arg
B
S
SL
XB
1
2
3
4

Figure 15. Main treatment likelihood ratio effects (logistic regression models) for reproductive efficiency traits (RET). Using the reduced model [2] a series of profiles were generated. For RETs based on reproductive status only a response of 1= pregnant and 0 = not pregnant is given on the left column. Values of reproductive status (next to left column) are given as coefficients. For RETs based on estimated embryo numbers go from 0 to 3. Some small values in the series may not be visible. Significance of effects are given in red as probability values (P). P4 exposure protocol: Long-CIDR 12-14 d (L), long-SMB 12. 14 d (L-Syn), non-synchronized (N), non-synchronized and non-fixed-time bred (NNT), short 5-6 d (S), and extra-long 24 d (XL). Breeding procedures are: Laparoscopically-aided intrauterine artificial insemination (LAI), transcervical AI (TrAI), and natural service (NS). PG600 dose level describes: None (0 mL), 1.75 mL, and 5 mL. Breeds were: Alpine (A), Angora (Ang), Boer (B), Spanish (S), Stiff-legs (SL), and crossbreds (xB).

CHAPTER V

EARLY PROGENY WASTAGE IN DAIRY AND MEAT/FIBER GOATS ARTIFICIALLY INSEMINATED AND NATURALLY SERVICED AT A FIXED-TIME FOLLOWING VARIABLE PROGESTERONE EXPOSURE AND DIFFERENT LEVELS OF ECG/HCG

Abstract

Prenatal and perinatal progeny loss (PPNL) of unselected cycling dairy and meat/fiber goat production phenotypes (n=870) was determined on the basis of non-return to estrus rates and atypical estrous intervals. Validation of the use atypical estrous interval to determine conception rate was performed with a subset of the breedings (n=84) used to determine pregnancy and embryo number at 21 d post breeding (dPB) by P4-RIA and at 45 dPB by P4-RIA and ultrasound imaging (UI). PPNL components were: embryonic mortality (EM), abortion (A), stillbirths (SB), dam C-section (C), maternal death (MD) and postpartum losses (PPL). Mean effects of first breeding goats (n=533) were analyzed for: a) Goat breed, b) Age, c) Parity, d) Breeding procedure (natural service, laparoscopically-aided intrauterine, and transcervical), e) Breeding number, f) P4 exposure (CIDR-G): 24d, 12-14d , 5-6d, and a control non-synchronized cohort, and g) use of PG600 at 0.0, 1.75 and 5 mL.

Records generated from two breeding/kidding seasons were used to fit polytomous logistic regression models. Analysis of agreement was performed by Bland-Altman methodology and using Cohen's (κ) for agreement and Bowker's test of symmetry. The global conception rate, 45-dPB UI pregnancy rate and kidding rate was determined to be: 72%, 60%, and 49%, accordingly. A 17% PPNL was found for the breeding group or 24% when considering only the group of goats conceiving. EM contributed from 50% (in Boer breed) to 91% (in Angora breed) of the total PPNL within breed losses and from 15% (in Spanish) to 27% (in Boer) in the case of SB. PPNL was the result of EM, SB, and A in 95% of females overall and PPNL was greatest in first time serviced females at 30% . The greatest loss was in primiparous goats at 47% followed by the nulliparous at 31% and multiparous at 23%. PG600 treatment affected CR ($P<0.03$) between the non-treated goats and those animals that received 1.75 mL giving an OR of 1.6. No differences ($P> 0.71$) were found between PG600 doses for the numbers of kids born per bred doe: 1.76, 1.77 and 1.81, respectively. Results of the agreement measures were ambiguous and, at times, contradictory as they lead to different conclusions in 60% of the comparisons.

Introduction

Improvement of goat production revenue entails managing emerging problems in an innovative manner and adopting new approaches to resolve long-standing challenges. One promising option combines increased production by upgrading herd genetics, while minimizing labor input through efficient use of labor-intensive herd reproductive management activities. Selected assisted reproductive technologies (ART) can be used as a tool to successfully satisfy both aims.

The biologic component of this objective can be fulfilled using artificial insemination (AI) with semen from commercially available high genetic merit sires; while, to take advantage of economies of scale when implementing an AI program, hormone based estrus/ovulation synchronization (E/OS) protocols combined with fixed-time AI control female reproduction physiology and reduce demand for labor.

Despite the potential benefits that could be attained with difficult-to-breed animals or breeding in non-typical scenarios, the adoption of ART techniques actually lower reproductive performance (RP) of a healthy, non-stressed[1] and reproductively sound goat receiving appropriate care.[2-5] Under the setting discussed, it is clear that there is a trade-off between the components that drive the profit equation; i.e., improvements in the gene pool and reduction in labor requirements combined need to outweigh diminished reproductive efficiency. The reason why ART reduces RP has multivariate roots and has been the subject of interest in many studies over the years.[1, 6-12] Nevertheless, the consensus is that, because of the low heritability of reproductive efficiency traits and even after accounting for pathological conditions,[8, 9, 13-15] reproduction can be profoundly

influenced directly, indirectly and by the interaction between environmental elements.[16, 17] Many factors tamper with the neuro-endocrine and paracrine/autocrine systems[18-27] resulting in modification of critical reproductive events.[28] In this study we hypothesize that E/OS protocols using short-term progesterone exposure and a concurrent combination of chorionic gonadotropins of human and equine origin interact adversely with fixed-time breeding increasing prenatal and perinatal loss.

Early progeny wastage in goats

Early progeny wastage in goats is estimated to be between 20 and 40%.[27, 29-32] The time when losses are sustained is not known with certitude. Loss time-points are critical because when breeding results are evaluated belatedly, the stage of development when prenatal losses occur cannot be determined precisely. Hence, targeted management action to improve reproduction is curtailed.

An embryo exists from syngamy through the time of maternal recognition and endometrial attachment, by this time a conceptus (embryo and placental membranes) is recognized until it becomes a fetus once differentiation of the embryonic/conceptus organs and placenta is concluded at approximately day 42 post fertilization, and/or when bone mineralization takes place to the time of parturition.[11] The pre-attachment period is the time from fertilization to the first loose attachment of the goat blastocyst to the endometrium on day 18 to19.[33, 34] The fetal period corresponds to the rest of the pregnancy until birth. Death during the pre-attachment period corresponds to early EM whilst that occurring during the remainder of the embryonic period is regarded as late embryonic loss. Failure to maintain a fetus during the remainder of pregnancy results in abortion. Birth of a dead kid is described as a stillbirth.

Documented attempts to characterize early progeny wastage in goats have occurred in few countries.[6, 35-41] Similarly, and with few notable exceptions,[42] estimates available addressing early goat wastage in North America have been generated with small samples not representative of the larger goat population and/or relevant environments. Therefore, it is crucial to determine the extent and time of occurrence of early progeny wastage in goat herds geographically located in areas where their production is relevant.

Although, under typical conditions, fertilization in ruminants approaches 100%[43] and, for this reason, considered an "all or nothing" proposition, in the case of goats, pregnancy losses may be partial or total. As depicted in Figure 1, prenatal loss consists of direct and indirect losses. Direct losses are the result of embryonic mortality and abortions. Indirect losses may occur as a consequence of incidental death of the mother or resulting from pregnancy complications requiring cesarean intervention resulting in complete or partial loss of the litter. Another important component of early progeny wastage is perinatal mortality which are losses occurring close to the time of kidding. Other offspring may die during the birthing process due to dystocia or other reasons. The last category of early deaths occurs shortly after a live birth and, for this reason, is termed early postnatal.

In summary, this study attempts to improve the information available on the extent and timing of early progeny wastage in goats of the Central Southwest and to evaluate the influence of the concurrent use of human and equine chorionic gonadotropins as part of hormonal E/OS protocols used for fixed-time breeding on prenatal and perinatal loss of goat progeny.

Material and Methods

Animals

This study was conducted using guidelines of the Animal Care and Use Committee at the American Institute for Goat Research (AIGR), Langston Oklahoma (*Lat*. 35.945° N *Long.* -97.255° W, 292 m.a.s.l.) during the reproductive seasons (September through January) of 2006 through 2008 and the subsequent kidding seasons. Each year from the beginning to the end of the breeding season daylight decreased from 12.8 h to 9.6 h.

The study included unselected mature and young goats representative of diverse production phenotypes: Dairy (Alpine), meat (Boer and Tennessee Stiff Legs), meat/fiber breeds (Spanish and Angora), and various percentage genotypic cross-bred (Boer × Spanish). Goats came from different existing parity categories and ranged from 1.4 to 11 y of age with an overall average age and ±SD of 4.1 ±1.5 y.

Animal Management

Details of animal management have been provided elsewhere.[44] Briefly, the Alpine herd consisted of non-lactating goats managed semi-extensively on Bermuda or Sudan grass as well as being placed in wheat pastures when fresh forage was available. Bucks had *ad-libitum* access to local prairie mixed grasses, Bermuda grass or Sudan grass hay as well as wheat hay.

This study also used non-lactating meat and fiber goats managed extensively on native Oklahoma mixed grasses and wheat pasture when fresh forage was available. All goats were provided fresh water and had free access to mineral supplement licking blocks. Goats received nutritional supplementation as needed based on body condition. All goats

remained under veterinary care and were treated regularly for internal parasites. Goats were cared for by trained farm personnel and had access to portable shelters.

Early progeny wastage terminology

In this study, as depicted in Figure 1, early progeny wastage has been characterized by the combination of prenatal and perinatal losses. Prenatal loss (PNL) is defined as pregnancy failure occurring due to direct causes such as embryonic mortality (EM) if death occurs from the time a zygote is formed until just before the time that the embryo is considered a fetus (approximately at 40 days) and abortions (A) when at least one fetus is lost from 41 days to a total time of 95% of the natural gestation length average of 150 -7 d -i.e. 143 d.

Progeny losses occurring as a consequence of incidental and/or accidental death of the mother (MD) or due to pregnancy or other complications which required removal of the fetus by cesarean (C) intervention, resulting in complete or partial loss of the litter, were classified as indirect losses. Perinatal loss (PL) describes animals born dead (stillbirths), deaths happening during the birthing process (D), and post-partum losses (PPL) occurring up to 24 h after birth.

Parturition management and kid care for the first 24 h of life

Intensively managed dairy herd

Management and care dispensed was no different than previous yearly routine kidding and nursery operations as established on AIGR research farm procedures.

Alpine breed females were brought into a ventilated and temperature controlled enclosed maternity facility approximately 10 d prior to kidding on the basis of dated breeding

records and the results of ±45 d post breeding (dPB) ultrasound imaging (UI) pregnancy diagnosis.

During their stay in maternity, does and doelings were fed a totally mixed non-lactating ration and had access to clean water via suction-activated water dispensers. Most births occurred in the presence of trained caretakers. Once pregnancy labor started females were under closer scrutiny and standard mother/kid delivery help was provided only after there were obvious signs of difficulties (e.g., incorrect kid positioning in the reproductive tract, excessive time in labor, diminished cervical relaxation, female became exhausted) for further obstetrical care animals were sent to Oklahoma State University Large Animal Clinic if necessary.

Farm personnel insured newborns were breathing normally and cleared placental membranes from nostrils, face and body when necessary. Umbilicus and hooves were dipped in 7% iodine solution and the rest of the body dried with clean towels if wet. Kids were separated from their mother at birth identified with ear tags, weighed, placed in a clean confined area heated by lamp when the ambient temperature was low. Feeding took place as soon as possible with colostrum obtained from does and doelings that had been pre-tested negative for caprine-arthritis-encephalitis virus, (i.e., CAEV). When feeding colostrum a mother's fresh colostrum was preferred when available, if the mother was CAEV positive, thawed/pre-heated, pre-harvested frozen CAEV negative colostrum was used. During the first 24 h of life kids were fed colostrum every 8 h.

Extensively managed meat and fiber herds

Management and care dispensed was the same as that provided in previous years as established in AIGR Research Farm procedures. Boer, Spanish, Angora, Tennessee-Stiff-Legs, and other different percentage cross-bred females were placed on clean pastures of Oklahoma native grasses several weeks prior to kidding. Pregnant goats had access to semi-permanent shelter, clean water, regular to good quality hay, and were supplemented with a commercially customized 20% protein goat pellet according to body condition.

Female goats were observed at least three times a day during day-light hours. When and if parturition labor was detected no interference was attempted unless females displayed similar complications as described above for the dairy herd. Upon kidding farm personnel insured newborns were breathing correctly and cleared placental membranes if necessary. Umbilicus and hooves were dipped in 7% iodine solution. Kids were identified with ear tags and ear notching and birth weight was recorded.

Offspring rejected by their mother were removed and raised as orphans in similar fashion as cared for Alpine kids (see above). Mothers were allowed to take care up to two kids. Progeny from litters in excess of twins were raised as orphaned kids. Attempts were made to graft excess kids to other singleton females kidding the same day.

Farm management was informed if kidding complications arose. When necessary females were brought indoors and delivery help was provided. Weak females were also brought indoors for closer supervision. If further obstetrical care was needed goats were sent to Oklahoma State University Large Animal Clinic; Stillwater, OK.

Estrus/Ovulation synchronization (E/OS) protocols

Details of synchronization protocols used in this study have been described elsewhere.[5] Briefly, all goats assigned to an experimental estrus synchronization protocol and breeding treatment group received on day 0 an intramuscular dose of 1 mL of Lutalyse® immediately after the luteolytic injection each goat received a P4 laden intravaginal device (CIDR-G®). CIDR's were kept in place for a pre-determined time period depending on which P4 exposure treatment group the goat was assigned to (see "Study variables" below). Twenty four h before P4 removal all synchronized goats received a pre-assigned dose of PG600® or, if in the control group, no gonadotropin was given (see "Study variables" below). Upon removal of the intravaginal insert a second i.m. luteolytic dose was given. Synchronized females were monitored for CIDR retention. If the P4 devise was expelled the goat was removed from the trial.

Breeding procedures

Breeding procedures used have been described elsewhere.[5] Briefly, three types of breeding techniques were used: transcervical artificial insemination (TrAI), laparosco-pically-aided intrauterine artificial insemination (LAI), and natural service (NS).

Goats were bred 48 to 50 h after removal of P4 using the randomly pre-assigned breeding treatment protocol. All TrAI procedures were carried out by two trained technicians each with the help of one other support personnel. LAI was accomplished by one trained technician with the help of two other support personnel, in both procedures goats were artificially inseminated whether or not overt estrus signs were observed. Frozen-thawed semen used for AI originated from a total of 26 sires from two commercial vendors. Each 0.50 mL straw contained 1×10^6 sperm.

Transcervical AI protocol (TrAI)

Standard TrAI procedures were used as described elsewhere.[45] TrAI protocol entailed an attempt to fully traverse all cervices. In some instances this involved applying additional light pressure on the AI gun and more extensive manipulation but avoiding, at all times, trauma to the reproductive tract. However, if unable to surpass all cervical rings, semen was deposited in the reproductive tract as far inside the cervix as possible or in the cranial portion of the vaginal vestibule.

Intrauterine laparoscopically-aided AI protocol (LAI)

Established LAI procedures were used.[44] All uses of scheduled drugs for the procedure were performed by a licensed practicing veterinarian or under his supervision. Summarizing, LAI protocol consisted on the following actions:

Goats were pharmacologically sedated and placed in dorsal recumbence on a 30º with respect to the horizontal, inclined cradle with the head down. Two sites at both sides of the midline were selected as entry ports. Local anesthetic was given at these sites for abdominal trocar/cannula puncture. Entry of both instruments was through two stab incisions made to an insufflated abdomen where CO_2 was delivered via an inserted Veress needle.

One of the inserted trocars was removed and the visualizing scope (25^{o}) inserted through the cannula. Semen preparation and LAI gun loading followed procedures similar to those used for TrAI as described previously.[45] The other inserted trocar was then removed and the inseminating gun, containing the thawed-frozen semen straw inside a plastic sleeve with a needle at its delivery terminal was introduced through the canula.

After inserting the inseminating needle in one of the uterine horns, half of a 0.5 mL straw was delivered. The other uterine inseminating site was selected and we proceeded as described previously depositing the remaining semen.

After insemination CO_2 was vented out of the abdomen through the trocars and instruments were then removed. Incisions were shut close using surgical staples. Goats were allowed to regain consciousness and were placed back on an isolated pen where they were supervised until fully recovered.

Natural service (NS)

Breeding by NS was used on two control groups (see explanation of controls used below under "study variables"). All naturally serviced goats were bred as penned-group females with one of 7 Boer or 8 Alpine farm-owned raddled bucks fitted with a breeding marking harness. Once bred goats were placed 5 to 7 d before their next scheduled estrus with clean-up bucks fitted with a breeding marking harness.

Early progeny wastage evaluation

For this study *early progeny wastage* is defined as the mortality occurring after the formation of a zygote until 24 h post birth. Pregnancy diagnosis is closely linked to the evaluation of prenatal and perinatal losses by virtue of being the fitting tool used to asses if a given mating produced progeny and whether or not it was carried to term. Except for the 45 dPB UI pregnancy diagnosis and its confirmation of pregnancy at kidding all diagnostics were based on indirect strategies of pregnancy diagnosis and/or the disruption of gestation.

As presented graphically in Figure 2, there are several direct and indirect methods that can be used to evaluate progeny losses. For completeness the graphic includes all strategies that potentially could be used in order to detect the approximate timing of these losses. This study has used a limited selection of these possibilities: Early luteal regression, non-return to estrus, atypical estrous cycle time length, 45 dPB UI, and germane reproduction records. On a randomly selected subsample, plasma P4levels at 22 and 45 d PB was used to validate non-return to estrus and/or atypical estrous cycle time length. Each given strategy of pregnancy diagnosis was implemented as follows:

Premature corpus luteum regression

Premature corpus luteum regression was assumed to have occurred if a bred goat came back to estrus 4 to10 d after breeding.[46, 47] With the presumed loss of P4 support, which in other studies has been shown to go under 1 ng/mL of blood plasma,[48] it was assumed that embryo(s) mortality had ensued.

Non-return to estrus

Bred goats that did not return to estrus 18 to 24 d on their next schedule estrus were reported as having conceived. Conversely, goats returning to estrus 18 to 24 days after having been bred were assumed to be open, i.e., not pregnant. The use of non-return to estrus rates were validated by a proof-of-principle approach using a subset of the experimental goats. That is, by using a subsample of bred goats (n=85) which were blood sampled 21 and 45 dPB for P4 analysis and by the use of UI at 45 dPB.

Atypical estrous cycle length

In this study the indirect method of pregnancy diagnosis as used previously[49] was modified as follows: We considered that conception had occurred if at least one embryo was physiologically recognized (i.e., maternal recognition of pregnancy took place. Thus, a biochemical pregnancy was indirectly documented) and this event presumably led to embryo endometrial attachment. As portrayed in Figure 3, this means that, after breeding on day 0, there was no return to the next scheduled estrus by the 17th and up to the 25th d. Or there was a return to estrus, after having been bred, in an uncharacteristic estrous cycle period (*i.e.,* > 24d to <39d for 1st cycle post breeding and > 24d to <39d, > 45d to < 60d days and >66d < 81d for second cycle post breeding).

Blood plasma progesterone (P4) level

As portrayed in the time-line of Figure 2, blood plasma was collected at two time points: days 21 and 45 post breeding (n=85 for each time period). Samples were obtained by jugular venipuncture, centrifuge-separated plasma harvested was frozen until commercially outsourced for P4 analysis by RIA at New Mexico State University, Las Cruces, NM, where P4 analysis was performed according to standard procedure.[50] In their hands the P4 assay yielded a laboratory CV of 4%.

In this study pregnancy diagnosis was determined according to characteristic plasma P4 levels of the estrous cycle as described elsewhere,[51-54] as well as P4 levels as a function of embryo number as determined in sheep[50] or in accordance to previous published data for goats.[55] In short, levels below 3 ng/mL were considered descriptive of non-pregnant goats, from 3 to 5.5 ng/mL was used to describe goats with one embryo and any goat with greater than 9.6 (5.5×1.75) ng/mL was assumed to have more than one embryo.

Ultrasound imaging

Details of UI procedural technique and validation for goat pregnancy detection and evaluation of embryo number (i.e., sensitivity, specificity, accuracy, relative error rate and predictive values) in different production phenotypes and parity categories has been provided elsewhere.[56] Briefly a mid-ventral external examination was conducted over a pre-clipped area of the posterior ventral abdomen, lateral and anterior to the udder using a portable real-time (B-mode) ALOKA SSD-500V (Aloka Co. Ltd., Japan) equipped with a 3.5 MHz linear array transducer (UST-934N) mounted on an external scanning device. To increase contact of the transducer with the animal's body the observation area was sprayed with alcohol. Transabdominal examinations were conducted by one trained technician. Does were placed on top of a milking stand with the head secured. Actual scanning was performed with the goat in standing position. Diagnosis was based on the recognition of any of the following: placentomes, beating heart, fetal head, thorax, limbs and fetal body movements if observed.

Study variables

Independent Variables

Three primary independent variables (treatments) were considered: Length of time of progesterone exposure, breeding procedure, and Dosage level of PG600. A description of each follows:

Length of time of progesterone exposure. Progesterone exposure time was based on the amount of time CIDR's remained in situ inside the vaginal vestibule. The following

treatments were used: N**one** (N), **short** (S)**:** 5 d, **long** (L)**:** 10 through 14 d and **extra-long** (XL): 24 d.

Breeding procedure. Breeding procedures used were: Trans-cervical artificial insemination (TrAI), laparoscopy-aided intrauterine insemination (LAI) and, natural service with penned group females (NSp). Except for one of the control groups (see explanation below) all goats, regardless of breeding procedure, were bred at a predetermined fixed-time.

As part of the breeding procedure comparisons we used two naturally-serviced control groups. a) A control group of naturally-serviced goats that were not synchronized (N) but were bred at a fixed-time 24 to 26 h after presentation of each individual doe's natural standing estrus (the timing selection was for the purpose of coinciding with the time of breeding of the synchronized goats at 48 to 50 h after CIDR removal), and b) A control group of naturally-serviced goats that were not synchronized and were not fixed-time bred (NNT). Therefore, this last control group was bred on an spontaneous estrus at a time determined by a given buck. In both control groups N and NNT does were removed from the breeding group as soon as a breeding was witnessed or a marked rump was observed.

Chorionic gonadotropin (PG600) dose level. The eCG/hCG hormone product (PG600) was intramuscular (i.m.) injected using a volume of: none, 140/70 units (1.75 mL) or 400/200 units (5 mL), respectively.

Blocking (covariate) variables

Secondary independent variables (blocking covariates) evaluated were: Goat breed as described previously: A, Ag, B, S, SL, and XB, TrAI technician (2), female age as a continuous or categorical ordinal variable (**1:** ≤3 y; **2:** >3 and ≤4 y; **3:** >4 and ≤5 y; **4:**>5 y), parity (nulliparous, primiparous and multiparous), and year of breeding/ kidding; 2006-7, 2007-8 and 2008-9 respectively.

Dependent (response) variables

In this study early progeny wastage was evaluated in terms of prenatal and perinatal losses (PPNL). The individual components of PPNL were: embryonic mortality (EM), fetal abortion (A), pregnancy losses associated with maternal death (MD), fetal loss due to premature cesarean-sections (C), stillbirths (SB), death during parturition (D), and death of kids shortly after kidding (up to 24 h); referred to as postpartum losses (PPL).

The analysis of main treatment effects on early reproductive wastage used EM as the early losses and all the remaining variables (i.e., A, DM, C, SB, D, and PPL) to describe late losses. EM was based on the all-or-nothing fertilization hypothesis and the independence hypothesis for embryo deaths biological hypotheses.[57] Mathematically EM was determined as the difference from goats diagnosed pregnant by the $NRR_{(18\text{-}24d)}$ method and the pregnancy status as determined by radio immune assay (RIA) $P4_{\text{-RIA}}$ at 21 d and at 45 d as well as the difference between $NRR_{(18\text{-}24d)}$ and 45 d transabdominal ultrasound imaging scan for pregnancy status.

The overall losses at any given time was obtained by calculating the difference between the method of pregnancy diagnosis × period of interest (i.e., 21d $NRR_{18\text{-}24}$, 21d $P4_{\text{-RIA}}$, 45d $P4_{\text{-RIA}}$, and 45d UI) and actual goats that kidded and litter size attained at term.

Statistical Analysis

Unless otherwise stated all data were analyzed using the computerized analysis system JMP v9.1.[58]

Experimental design

A prospective clinical study using randomized control trial was implemented. Some reproductive data was analyzed retrospectively.

Sample size

Goats that did not become pregnant to a particular assigned breeding procedure at the first breeding attempt were re-assigned to be bred a 2nd, or 3rd or 4th time by means of natural service. For this reason a total of 879 breeding records, accumulated over two years (2008/2009), were reviewed to determine overall prenatal and perinatal loss evaluation descriptive statistics. Considering the final breeding group database (n=879) the percentage of unique observations was 40%. In other words, some goats were evaluated more than once (*i.e.,* the goat was rebred by NSp if open in the same '08 year, or the same goat was used in the subsequent '09 breeding year). Of these 879 goats exposed to males, 9 females assigned to be bred by NS did not come in estrus, hence only 870 were bred.

Treatment effects were analyzed using only the data generated by 533 first-breeding goats. Of the 533 first-breeding goats, 416 (78**%)** observations were unique.

Proof-of-concept concerning the use of non-return rates and irregular estrous cycle length to determine embryo mortality used a sub-sample group of 84 (17%) randomly chosen goats within the Alpine and cross-bred goats (Boer/Spanish) belonging to the 2008 breeding season.

Statistical model

Reproductive continuous numeric variables such as P4 blood serum levels were analyzed with a general mixed linear model. Variables containing binary states (*i.e.,* pregnant or not pregnant, kidded or not kidded) were analyzed using a logistic regression model as previously described[59, 63]

$$\hat{P} = \frac{e^{b_o + b_1 x_1 + b_2 x_2}}{1 + e^{b_o + b_1 x_1 + b_2 x_2}}$$

where,

b_o represents the Y axis intercept, b_1 is the regression coefficient, and X_1 is the predictor. Both b_o and b_1 were estimated by the maximum likelihood method (JMP, 2011). As described earlier.[60] The null hypothesis underlying the overall model considers all regressors as being equal to zero.

Statistical model evaluation

The appropriateness off all statistical models used was based on how well the data fitted to a given model using the lack of fit (LOF) statistical methodology.[63] When more than one statistical model was considered the Akaike's information criterion (AIC), which takes into account the statistical goodness of fit and the number of parameters that have

to be estimated to achieve a given degree of fit, was used as an index to choose between competing models.[64] Where, lower values of the AIC index indicate the preferred model, that is, the one with the fewest parameters that still provides an adequate fit to the data.

Initially data was fitted to a semi-saturated model which only included the interactions of interest. Response variables that did not significantly reduce the model's variability were dropped from the model.

Odds ratio

The interpretation of results of the likelihood ratio tests and between parameter estimates was accomplished using the odds ratio for both categorical and continuous predictors. Analysis of differences between proportions, in terms of comparative outcome of several 2×2 contingency tables for different classes of the covariate of interest, were also performed by means of odds ratios [61,62] as follows:

$$\boldsymbol{OR} = \frac{p_1/(1-p_1)}{p_2/(1-p_2)} = \frac{p_1/q_1}{p_2/q_2} - \frac{p_{1\times}q_2}{p_{2\times}q_1}$$

Where, an odds ratio (OR) of 1 indicates that X and Y are independent and that the probability of an event can be expressed in terms of their marginal probabilities. That is, the condition or event under study is equally likely to occur in both groups. An odds ratio greater than 1 indicates that the condition or event is more likely to occur in the first group. And an OR less than 1 indicate that the condition or event is less likely to occur in the first group compared to the probability of occurring in the 2nd group.

When necessary for ease of explaining results conversion from an OR to probabilities, and *vice versa*, was performed as follows:

$$OR = \frac{\text{Probability}}{1 - \text{Probability}} \qquad and, \qquad Probability = \frac{\text{OR}}{1 + \text{OR}}$$

Statistical inference for OR significance between length of P4 exposure, within breeding procedure, were analyzed by use of the Chi-square (χ^2) test with P values corresponding to two sided tests and one degree of freedom as described in JMP v9.1.[58]

$$\chi^2 = \frac{((|\text{Observed Frequency} - \text{Expected frequency}|) - 0.5)^2}{\text{Expected frequency}}$$

Agreement Analysis

Continuos variables

Analysis of agreement between 21d $P4_{\text{-RIA}}$ and $45d_{\text{4-RIA}}$ data, with a null hypothesis (*Ho)* test that bias is zero, was carried out by Bland-Altman graphical approach as previously described.[65-69] Because the value ranges were low, bar charts were used to enhance the data distribution of visually descriptive Bland-Altman plots as suggested.[68]

Agreement analyses and graphs were obtained using the Analyse-it program version 2.24 (Analyse-it Software, Ltd.; Leeds, United Kingdom).[70]

Discrete variables. Analysis of agreement between the different pregnancy diagnosis methods, which contained categorical outcomes (pregnant or non-pregnant) at each period of interest was determined using Cohen's agreement statistic (κ) to match levels across the two categorical variables[71] as well as by using Bowker's test for symmetry.[72]

Cohen's (κ) coefficient equals 1 when there is complete agreement of the raters[58]; the null hypothesis tested is the existence of no agreement between raters. In the case of "Bowker's test of symmetry", the null hypothesis is that the probabilities in the square table satisfy symmetry, or that $p_{ij}=p_{ji}$ for all pairs of table cells.[58]

Results

Overall diagnosed pregnancy and achieved kidding rates

The global average 45-day post-breeding (dPB) UI diagnosed pregnancy rate (PR) and the corresponding kidding rate (KR) at term for those UI scanned goats was 60% (398/666) and 49% (343/666), accordingly.

Factors that affected PR and KR were the result of calculations arrived at using data generated from goat RP which included germane categories to this study. That is, global statistics were obtained using the following comprehensive model:

[1] ***Response variable*** **=** Breed + Age group + Parity + Breeding procedure + Number of breedings + P4 exposure + PG600 dose + Breeding/ kidding season + Age × PG600 + Parity × PG600

where,

the response variable was either the number of goats diagnosed pregnant at 45 dPB or the number of goats that kidded at term.

Statistically significant results concerning the effect of E/OS protocols on breeding procedures were discussed in the previous Chapter and will not be repeated here.[44] After determining that the breeding/kidding season did not influence pregnancy ($P>0.76$) or kidding ($P>0.269$) a model without this factor was used.

45 dPB UI diagnosed pregnancy rate

As shown in Table 1, data fitted to a logistic model which included all the remaining independent variables as described in model [1], revealed that only PG600 and the interaction between PG600 and parity did not influence the overall PR at ($P>0.81$) and

(P>0.172), respectively. Breeding number and the interaction between Age group × PG600 were close to being statistically significant, (P<0.0532) and (P<0.062), respectively. In figure 4 PR is shown as bar graphs according to each of the 5 breeds and one mixed genotype of all females used in this study (i.e., goats bred). PR's differed among breeds (P<0.0001) and ranged from 95% for Spanish goats to 30% for the Tennessee Stiff-leg breed.

Attained kidding rate of goats UI scanned

Using a similar logistic model to model [1], variables that did not contribute significantly to explain the model's variation were sequentially dropped from consideration: kidding season (P>0.269), parity × PG600 (P>0.165), age group × PG600 (P>0.168), and breed (P>0.101).

Application of he reduced model indicated that the number of goats kidding was influenced by: age group (P<0.006), parity (P<0.003), P4 exposure (P<0.009), breeding procedure (P<0.0001) and number of breedings (P<0.0001). Retained in the model was PG600 which had no influence (P>0.289) on the number of kidding females.

The actual realized parturition performance for each breed can be seen graphically in Figure 5. Panel [A] describes a total of 247 females that kidded at term (using exclusively the previously UI-scanned animals; n= 666). This means that a 43% kidding rate was attained in this goat group.

Also in Figure 5, panel [B] depicts kidding rates when all the bred females are taken into account (n=870). No difference (P>0.05) was found between these two analysis groups. Such that, the global kidding rate was calculated to be 44% (383/870).

Overall conception rate (CR), prenatal and perinatal losses (PPNL)

During the two breeding/kidding seasons accounted for in this study, there were a total of 870 recorded breedings that resulted in 628 goats conceiving, this represents a 72 % CR. Conception was estimated by various direct and indirect approaches as explained in "Material and Methods". Of the females that were bred, 150 gestations or 17% ended in death while progeny were *in utero* or shortly after birth up to the first 24 h of life. If the same PPNL statistic is calculated on the basis of only goats having conceived then PPNL reached 24%.

As can be seen in panel [A] of figure 6, the distribution of loss instances documented was not homogeneous across mortality category cases. Embryo mortality at 64% with a $CI_{95\%}$= (52, 78) of the total PPNL was the main cause of PNL (see Table 2 panel A) followed by stillbirths at 19% with a $CI_{95\%}$= (13, 28) and abortions at 11% with a $CI_{95\%}$= (7, 18). Embryonic mortality, stillbirths and abortions together were responsible for an estimated 95% of the global early progeny wastage. For statistical analysis purposes the last three groups were combined to constitute only one category. As a result of the new grouping the last three categories (C, DP, and PPL) formed a new class which contained 5% with a $CI_{95\%}$= (2, 11) of the observations as given in Table 2, panel [B].

Although cases of dystocia were documented throughout the extent of this study the incidence was minimal in the case of meat and fiber herds at less than 1.5%. However, dystocia was more relevant for the dairy animals which had 6.4% of goats having difficult deliveries at parturition. Nonetheless, as documented in Table 2, panel [A] none of these problematic gynecology events lead to newborn deaths. Therefore, the dystocia category was not considered further and for statistical analysis purposes (i.e., greater

number of experimental units per group classification analyzed) the last three groups were combined to constitute only one category. As a result of the new grouping the last three categories (C, MD, and PPL) formed a new class which contained 5% with a $CI_{95\%}$= (2, 11) of the observations as given in Table 2, panel [B].

When total prenatal and perinatal (PPNL) loss data was fitted to a logistic model structured as follows:

[2] ***PPNL*** = Breed + Age + Breeding number + Parity category + AI technician

where,

age was analyzed, in turn, both as a continuous or as a categorical independent variable, each in a different model, breeding number corresponding to whether or not there was a 1^{st}, and 2^{nd}, and 3^{rd} and 4^{th} breedings. Using model [2] only breed ($P<0.0005$) and parity ($P<0.01$) had a significant statistical influence on the overall PPNL, as reported next:

Overall prenatal and perinatal losses as a function of breed

The purported global early progeny loss is given in Figure 7 both as the total number of observations in the ordinate axis, which coincides with the top of each category in the bar graph, and also as a percentage of total losses per breed in which case the percent number is placed on top of each of the bar graph category columns.

Figure 7 is counter intuitive because although a longer bar column implies greater number of observations in a given pre/peri-natal loss category, compared to the other loss categories and their respective bar columns, it cannot be inferred from this information that a particular category had greater loss based on the total frequency of

cases recorded; more fittingly, the value needs to be evaluated as a ratio, i.e., relative to the number of goats in each breed.

The percentage value, which can be used to compare across breeds, has been included on top of each bar graph column of Figure 7. For example, the last genotypic category of x-bred goats shows that the total number of embryos lost were 31(following the dotted line from the top of the column to the ordinate axis) which represents 60% of the total 52 x-bred embryos lost during both reproductive seasons. Whereas, graphically the Angora breed is portrayed with a shorter column bar compared to all the other bars in the graph because, numerically, Angoras had the least embryos lost (n=10), yet as a percentage of its breed, Angoras had in fact the greatest embryo mortality reaching 91%.

As portrayed in Figure 7, early progeny wastage occurred in all 6 breeds considered during the two breeding seasons studied (n=870) and the distribution of losses among breeds was different. EM contributed from 50% (in Boer breed) to 91% (in Angora breed) of the total PPNL within breed losses and from 15% (in Spanish) to 27% (in Boer) in the case of SB.

Overall embryo mortality according to breed

The total number of embryos detected was 669 for a total of 604 goats exposed to bucks or artificially inseminate. As given in Table 3, the most prolific goats were the T. Stiff-legs and Alpine breeds. Least prolific was the Angora goat breed. The distribution of pre/peri-natal losses (PPNL) components in reference to the associated breed is severely skewed because of the sporadic early progeny losses among some of the breeds (see

Table 4). Hence, valid mean comparison involving breed is only justified for EM (dotted fill pattern columns in Table 4).

The number of embryos that were included in Table 3 was also used in Table 4. However, to determine the prenatal component loss according to goat breed the fluid vesicles counted as embryos in Table 3 were not taken into account on Table 4. As tabulated in Table 4, compared to the other goat breeds the T. Stiff-leg breed presented the greatest PPNL (50%). T. Stiff-legs differed from all other breeds ($P< 0.008$), except Angora ($P= 0.8172$) which had the 2nd to the greatest EM with 42%. This probability entails, for the T. Stiff-leg breed, an OR from 3.7 to 7.5 times greater likelihood of embryonic losses depending on the breed to which it's compared to.

Angoras, with a 42% PNL, differed ($P< 0.02$) from the rest of the breeds in EM (38%), except with the T. Stiff-leg as explained above. Crossbred goats had a 20% PNL, and their 12% EM differed ($P< 0.0002$) only from the T. Stiff-leg and Angora breeds. Boer goats had the least PPNL at17% and also the smallest amount of EM at 9%. Their EM differed only from Angora and TSL goats. These percentages translate to an OR of 6.6 and 7.5 times greater likelihood that these breeds would have EM losses, respectively as compared to the Boer breed.

Overall prenatal and perinatal losses as a function of parity

The overall prenatal and perinatal losses as a function of parity, is graphically portrayed in Figure 8. As explained previously, this bar graph which describes PPNL according to each parity category, is counterintuitive as well, the height of individual bars reflects total number observed while the percentage value on top of each bar column represents the specific PPNL item contribution for a given parity category.

The greatest number of embryos lost was attributed to P goats at 47% (70/150) followed by the N at 31% (46/150) and M at 23% (34/150). The distribution of progeny losses within each parity level was also different going from 83% in EM for the N goats to 50% in the M. Stillbirths attained a maximum value within parity of 29% which was observed for the P goats while the N only had a 9% contribution to the PPNL of the group. Abortions within each parity category occurred from 6.5% for the N to 18% in the M goats.

Main treatment effects on the time of early progeny wastage occurrence

For standardization purposes and to eliminate confounding effects on prenatal and perinatal loss resulting from introducing unknown sources, main treatment effects were evaluated using exclusively data generated by first-time bred goats (n=533). The streamed lined nominal logistic fit model for early progeny wastage (PPNL) used was:

[3] ***PPNL*** = Breed + Breeding procedure + P4 exposure + PG600 + Parity

PPNL data fitted model [3] yielded a calculated χ^2 for LOF of 41 (P>0.670). With the results obtained using model [3] one is able to explain much of the time period variation (early vs late) occurring during progeny wastage in the sampled goats (P<0.0001). Of all the potentially influential variables considered, four turned out to be determinant. Goat breed had the greatest influence (P<0.0002) of all the main treatment effects followed by the parity category where goats were classified into (P<0.014), breeding procedure used (P<0.034) and the length of time a goat was exposed to P4 in the E/OS synchronization protocol (P<0.044). The effect of the concurrent use of equine and human chorionic gonadotropins was seen with considerably less significance (P<0.137).

Individual comparisons between the significant OR's obtained and their $CI_{95\%}$ are given in Table 5. The interpretation of these main effect results follows:

Breed

Spanish goats have a greater likelihood ($P<0.0005$) of having early losses than late losses as compared to Alpine and Boer breeds. Spanish goats also have a slight likelihood of having earlier than late PPLN ($P<0.060$) when compared to Stiff-Legs and x-breds but the biological significance of the odds ratio is so small that is irrelevant.

When compared to Alpine goats cross-bred goats are almost 14× more prone ($P<0.01$) to have embryo mortality than losses which occur later. On the contrary, the Alpine breed would be more likely to have late progeny wastage in the form of abortion(s) or stillbirths. Compared to the Boer breed, x-breds are almost 5× more likely to have early than late losses ($P<0.04$).

Progesterone exposure in the E/OS protocol

Progesterone exposure did not have any influence connected to the timing of early progeny wastage occurrence. However, comparisons between the two control groups of goats (those not receiving a hormonal treatment) showed that females that were naturally serviced and were not hormonally synchronized or bred by a fixed-time method (NNT) were almost 17× more likely to have early prenatal losses than goats that were also naturally serviced but were fixed-time bred (N).

PG600 dosage level in the E/OS n protocol

Goats receiving 5 mL of PG600 as part of their E/OS protocol, had 7× greater likelihood ($P<0.054$) of having early pregnancy losses than goats that received 1.75 mL PG600.

Breeding procedure

Goats bred by TrAI were almost 14× more likely of having early losses than goats bred by NS. The comparison between goats bred by NS and LAI breeding was significant ($P<0.016$) but the OR effect was in practice biologically and, from a reproductive management perspective, irrelevant due to its small magnitude and the ensuing herd impact it would have.

Parity category

Nulliparous goats were 12× more likely ($P<0.004$) to have early losses than multiparous goats that had, on average, more losses after 40 days.

Prediction profiles of early and late progeny wastage losses

Depicted in Figure 9 are five predicted profiles (of 810 possible main treatment effect combinations) of the distribution of early and late progeny wastage as a function of main treatment effect changes corresponding to model [3]. The results of these effects follow:

First panel of Figure 9: Typical goat

For the first row (panel group) in Figure 9 the independent main treatment variables selected were those intended to reflect, as close as possible, a natural state of the most typical goat condition as allowed by the limitations within this study. Such a "control" goat was characterized (going from left to right) as belonging to the most numerous group of goats, i.e., the x-breds, with a reproductive history of multiparity, being bred on a spontaneously expressed estrus by natural service at a time determined by the buck. This putative representative average x-bred goat, if it has progeny losses, then it would be expected that a herd would characteristically have 26% of the progeny losses early

(i.e., up to day 40 of gestation) with the bulk of the offspring losses (74%) taking place late in gestation.

Second panel of Figure 9: Effect of P4

When P4 exposure is analyzed keeping the remaining variables at their initial values, goats not synchronized but fixed-time bred (N) and goats receiving the extra-long P4 exposure (XL), are predicted to have a greater number of goats (97%) sustaining losses late in gestation compared to the number of deaths of untreated control goats. When goats receive the short P4 exposure it is predicted that all goats having early progeny wastage would experience exclusively early losses.

Third panel of Figure 9: Effect of PG600

Going from no treatment with PG600 to a dose of 1.75 mL changed (non- significantly) the relative levels of losses in Angoras. Additionally the use of PG600 interacted with low levels of P4 exposure to reduce the number of goats experiencing early losses.

Fourth panel of Figure9: Effect of breeding procedure

Both AI'ed groups (LAI and TrAI) are predicted to have more females experiencing early losses at 78% and 83%, respectively than the 26% occurring early in gestation as predicted by the model for goats bred by natural service.

Fifth panel of Figure9: Effect of parity category

When the remaining variables were kept at their original value, a change in parity category shows that multiparous goats would be predicted to have mostly losses after the 40^{th} d, and that in both N and P goats losses would occur before day 40 at 81% and 55%, accordingly.

Effect of chorionic gonadotropin dosage level

Litter size

The influence of concurrent use of eCG and hCG at two different dosage levels, as part of the hormonal protocol for E/OS and in accordance to the breeding procedure used, was of interest to establish whether assisted reproductive techniques, such as fixed-time TrAI and LAI were detrimental to the realized litter size at term.

The effect of breeding procedure on reproductive efficiency traits has already been discussed elsewhere[44] and will not be repeated here. Briefly in all cases fixed-time TrAI gave the lowest percentage of pregnancies (i.e., 24%). For this reason it was deemed of importance to analyze the potential involvement of PG600 on pregnancy rate.

In the context of different levels of PG600, as can be seen in Figure 10, despite the changes in the proportion of kids born as a result of different breeding procedures, PG600 did not influence reproductive performance when the efficiency trait measured was prolificacy. No differences ($P > 0.71$) were found between the 0, 1.75 and 5 mL of PG600 for the number of kids born per bred doe: 1.76, 1.77 and 1.81, respectively. Figure 10 also shows that litters of size 4 and 5 were obtained when PG600 was given. The response to PG600 appeared to be favorable particularly for the animals that were not inseminated. Going from 58% triplets to 82% and 75%, with twins the percentage went from 76% to 76% and 80% and singletons went from 68% to 64% and 75%.

The total prenatal and perinatal losses considering goats only subject to a first breeding (n=533), within PG600 treated and untreated groups, was calculated to be 19% (see

Table 6). Embryo number at conception shows that the overall first service conception rate, adjusted for fertile females that underwent both PNL and PL, was 61%.

As presented on Table 6, first service CR was highest (66%) for goats that did not receive PG600 compared with 60% CR for goats receiving 5 mL and 55% CR for goats that were treated with 1.75 mL of PG600. However, only the difference of 11% units between the non-treated goats and those animals that received 1.75 mL of PG600 was significant ($P<0.03$) giving an OR of 1.6; the other comparisons, that is, no PG600 vs 1.75 mL and 1.75 mL vs 5.0 mL resulted in a 1.3 OR ($P>0.335$) and 1.2 OR ($P>0.376$).

Effect of chorionic gonadotropin dosage level on prenatal loss components

With the exception of embryo mortality, all other individual trait components of PPNL could not be shown to be influenced by PG600 dosage level. Shown on Table 6, is the effect of PG600 dose on PNL documented for the 11% unit difference between goats receiving 1.75 mL of PG600 and goats in the control (no gonadotropin) treatment ($P<0.035$).

Progeny mortality

Over the time course of the study, a total of 575 embryos were produced by 324 females from a total of 533 that were bred (see Table 7). The original UI estimate for reproductive status was 247 pregnant of 439 scanned/mated goats or 56% PR. However, only 49% (214/439) of this scanned/mated does eventually kidded.

Even though at time of kidding litters of quadruplets and quintuplets were documented these had not been detected through UI scanning. In all, a total of 78 single fetuses, followed by 154 sets of twins and 18 sets of triplets, for a grand total of 438 fetuses were

detected. Accounting the number of offspring born showed a discrepancy with number of kids born (i.e., litter size).

EM turned out to be higher ($P<0.009$) for goats treated with 1.75 mL of PG600 (28%) compared to untreated goats (13%). However, at 28% EM was not different ($P>0.48$) from the 22% EM of goats that received 5 mL of PG600.

Effect of age on early progeny wastage (PPNL)

We now consider the effect of the age of goats on prenatal and perinatal losses (PPNL). The prenatal loss data was fitted to a minimal linear model described as:

[4] ***PPNL*= Intercept + Age**

The resulting data scatter graph is given in the upper [a] panel of Figure 11. Regarding the effect of age on prenatal and perinatal losses, fitting the data to this model resulted in a difference between the full and reduced model that was insufficient to yield statistically significant effects ($P>0.2119$).

As depicted on the lower panel [b] of Figure 11, when the PPNL data was fitted only to the first three early progeny loss categories (i.e., EM, SB, and A), responsible for 95% of the PPNL, to a logistic model given by Log(p/1-p) the following probability statements resulted:

a. **P(Abortion | Age)=** $\beta_o + (\beta_1 \times \text{Age}) = -1.39 + 0.193 \times \text{Age}$
b. **P(Embryo mortality | Age)=** $\beta_o + (\beta_1 \times \text{Age}) = 0.74 + 0.071 \times \text{Age}$

That is, for the log odds of aborted/stillbirth (a) and embryo mortality/stillbirth (b):

The OR for a 7-year increase in age is = $e^{0.193\times7} = 2.72^{1.351} = 3.9$ for abortion. This means that an increase of 7 years of age in the dam (estimated goat reproductive life) would increase to almost 4× the likelihood of a pregnant female experiencing abortion rather than stillbirths. Additionally, an increase in the same number of years would mean an OR = $e^{0.071\times7} = 2.72^{0.497}$ which results in 1.64 × the likelihood of an older doe having embryo death compared to stillbirths. In summary, older does that suffer early progeny losses are more likely to have abortions rather than stillbirths and less likely of having embryo losses rather than stillbirths.

Thus, given that a PPNL loss occurs the probability of the type of loss involved depends on the age of the mother. Although in all three cases there appears to be a tendency for abortion to increase with age relative to the other category loss contribution to which it is compared. EM appears to be steady throughout the range of goat ages studied, and the probability of stillborn also decreases with age. The results explained by model [4] (LOF χ^2= 147; P>0.40) turned out not to be statistically significant (P>0.71).

Agreement Analysis

Several comparisons between different measures for pregnancy status and embryo number diagnosis at different time points were performed to determine the agreement between these statistics. Only the comparison between levels of blood plasma P4 at 22 and at 42 dPB involved numeric continuous variables that permitted legitimate Bland-Altman methodology analysis. The remaining comparisons involved categorical variables that were analyzed with Cohen's kappa (κ) and Bowker's test for symmetry.

Bland-Altman analysis for blood plasma P4 at 21 and 42 dPB

As presented in Figure 12 the agreement plot shows that data on the diagonal is quite dispersed on either side of the line of equality, visually suggesting lack of agreement between the hormone levels. A -0.54 bias with a $CI_{95\%}$ (-1.51 to 0.42) ($P>0.267$) was calculated. Therefore, no bias was present between the blood plasma sampling time points. However, an analysis of variance (ANOVA) on the same data indicates that the means of both timed samples were different ($P<0.02$).

The difference plot also shows that six of the P4 values were ±2 SD away from the mean difference. Blood plasma P4 had a normal distribution where almost 50% of the hormone values were within ±5 ng from the mean difference of cero between samples taken on day 21 after breeding compared to samples obtained 42 dPB.

Agreement between pregnancy diagnosis measures for categorical results

A half matrix for Cohen's agreement (κ) and Bowker's test of symmetry is given in Table 8. The results of the measures of agreement were ambiguous and, at times, contradictory as they lead to different conclusions in 60% of the comparisons.

Agreement between non-return to estrus and other pregnancy diagnosis measures

The conclusions regarding pregnancy diagnosis on the basis of non-return to estrus method agreed with the pregnancy levels established for P4 at 42 dPB ($P<0.0190$), with the 45 d UI results ($P<0.0001$), and at kidding time ($P<0.0001$). However, non-return to estrus did not agree with the P4 values obtained at 21 dPB ($P>0.221$).

Agreement between $P4_{-RIA}$ blood plasma levels at 21 dPB and other measures used to determine pregnancy

As can be seen in Table 8 none of the other measures used for pregnancy diagnosis agreed with the P4 blood plasma threshold level determined by RIA and selected to indicate a goat was pregnant at 21 dPB. Whereas Bowker's test for symmetry showed that the 21 dPB P4 level was in concordance with the results obtained for blood plasma P4 at 42 dPB and with actual kidding results.

Agreement between $P4_{-RIA}$ blood plasma levels and Ul at 21 dPB or at kidding

Both, UI at 45 dPB ($P<0.05$) and the final results at kidding ($P<0.026$) were in agreement regarding whether females were pregnant or open. In the first comparison between P4 blood plasma at 42 dPB and pregnancy diagnosis aided by UI scans at the same time point Bowker's test for symmetry turned out different ($P<0.01$) than the conclusion reached by using Cohen's agreement (κ).

Agreement between 45 dPB Ul pregnancy diagnosis and kidding results

As can be seen in Table 8, Cohen's agreement (κ) leads to a highly significant rejection of the null hypothesis of absence of agreement ($P<0.0001$) whereas Bowker's test for symmetry between both categorical variables is barely accepted ($P>0.059$).

Agreement between diagnostic procedures to determine number of progeny

Agreement between $P4_{-RIA}$ blood plasma levels at 21 and 42 dPB

There was a discrepancy between the measures of agreement (Cohen's κ and Bowker's test for symmetry), concerning the comparison of the number of embryos estimated on the basis of P4 levels on day 21 and those estimated on the basis of P4 levels 42 dPB.

As presented in Table 9, when Cohen's (κ) was used to determine the level of agreement between P4 blood plasma levels at 21 and at 42 dPB the number of calculated embryos was different ($P<0.01$) between 21 dPB blood plasma P4 and 42 dPB, between 21 dPB collected blood plasma P4 and 42 dPB UI, and between 21 dPB blood plasma P4 and litter size at kidding. Whereas using Bowker's test for symmetry the conclusion is that both times of blood plasma collection do not satisfy symmetry.

The number of embryos diagnosed at 42 dPB was different from the number of embryos estimated by 45 dPB UI ($P<0.004$) and also not the same to the actual number of progeny born at term ($P<0.002$). Application of Bowkers' test for symmetry leads to conclude that both time points generated symmetrical results.

Finally, the number of embryos diagnosed by UI at 45 dPB was not the same as the number of progeny born at term ($P<0.001$) and ($P<0.067$).

Discussion

Commercial goat producers aim to increase profit margins of their operations. To that end they have adopted various ART's that offer several advantages over traditional breeding methods by reducing labor input and increasing access to premium genetics. However, despite the potential gains that could be attained, all the technological approaches have, so far, tended to decrease reproductive performance. Furthermore, implementing E/OS protocols have been shown to negatively intensify inadequate results when combined with fixed-time breeding.[44]

The reduction in effectiveness can be due to many factors and, in this study we explored the possibility that in large part it may be due to increased losses after fertilization, through early embryo and fetal mortality.

Representative statistics for comparison concerning caprine prenatal and perinatal losses in the U.S. is incipient at best. Much scarcer yet is data generated from the mid-central South-Western area which is home to the largest regional goat population.
Hence, in this study there was interest in evaluating the levels of early progeny wastage in typical goat breeds and to determine the extent and time when these losses occur as a result of using a specific type of hormonal E/OS protocol combined with fixed-time breeding. This study presents evidence showing that the extent of early losses is considerable and demonstrates that the timing of the early progeny losses are not an imperative absolute, but rather they depend on the particular independent variable(s) considered (i.e., breed, age, parity, breeding procedure, E/OS protocol, and the number of breedings).

Early progeny wastage

A portion of the PNL, particularly those associated with EM occurring under normal and stress-free conditions, are deemed to be unavoidable and are called “basal EM”. In fact[73] postulated that basal embryonic death may be a "way of eliminating unfit genotypes at low biological cost". Nevertheless, it is now generally recognized, that identifiable factors exist which cause embryo death rate to rise beyond this inadequately defined basal limit threshold.

Overall diagnosed pregnancy and achieved kidding rates

Although the global average 45 d UI diagnosed pregnancy and realized kidding rate, was reported to be, in this study, 60% and 49% accordingly. The difference between diagnosed pregnancy and realized kidding rate does not convey the level of global early prenatal mortality because: a) UI was performed only to monitor performance results to the first breeding in order to evaluate main treatment effects. Nonetheless, a global measure, by definition, must include all breedings and all kiddings not just what transpires after the first event. That is PR at 45 dPB excludes pregnancy and mortality determined by other means, and b) the difference between the diagnosed pregnant females and the progeny born ignores early embryonic mortality that could have occurred in the first 3 weeks after fertilization has taken place.

Under the above conditions PR remains at 60%, (398/666) no matter how many breedings took place because the determining factor for inclusion in the calculation is the fact that they had to have been UI scanned. Whereas, the global kidding rate, turned out to be 44% (383/870) because it included a larger data base.

Both global PR and KR were influenced by: breed, age group, parity category, the procedure used to breed animals, and the number of breedings. Breed had a highly significant effect on PR but not on KR. This result is expected since PR would be highly associated with ovulation rate that differs between goat breeds, but the effect would be "diluted" until the time of kidding by the very factors that affect prenatal losses differently to different species.

Insofar as the E/OS protocol components are concerned, P4 was influential only between control goats that were not synchronized but one of the control groups was fixed-time bred. This result shows the effect of fixed-time breeding but offers no utilitarian application since naturally serviced goats, even if E/O synchronized would not be fixed-time bred as this action would be impractical. PR was influenced by the interaction between age and PG600 dose level. However, the same interaction was not influential for KR.

Methodology used for conception and pregnancy diagnosis

The initial methods used to determine and quantitatively describe PNL have largely been a pregnant female not showing characteristic behavior of sexual receptivity on her next scheduled estrus 18 to 24 d after breeding (non-return to estrus; NR), but rather as late as 35 to 50 d. Does have been observed to have a normal 21 ±4 d length if no fertilization occurs or if the embryos die before day 15 when maternal recognition of pregnancy occurs in goats.[74] For this reason, when using the NR approach, EM before maternal recognition occurs cannot be discriminated from unsuccessful fertilization. This problem can be circumvented by use of direct observation of ovarian ovulation sites (i.e., laparotomy, endoscopy or ultrasound), hormonal analysis or CL maintenance with the

caveat that the earliest embryos can be detected is day 20th with P4 analysis, day 30th by transrectal sonography and day 40th using transabdominal sonography.

The approach used in this study to determine whether or not a female conceived deserves specific mention. The use of non-return to estrus and atypical estrous intervals has been used by other researchers[49]. Here, as proof of principle, we have compared the PPNL results generated by 85 goats at days 21 and 45 by such an approach with the conclusions concerning pregnancy arrived when plasma P4 levels are monitored. The lines of support evidence clearly indicate that analyzing breeding records yields the same results as those evidenced by the endocrine approach.

Overall conception rate and distribution of prenatal and perinatal losses

Using the different behavioral and physiologic methodology described above it was estimated that a global CR of 72% (628/870) was attained and by monitoring pregnancy at different time points and documenting fetal losses the global early progeny wastage of goats that conceived was calculated to be 28%.

Clearly, as can be seen in panel [A] of figure 6, the distribution of loss instances documented was not homogeneous across mortality category cases. Embryonic mortality, stillbirths and abortions together were responsible for a calculated 95% of the global early progeny wastage in goats.

The overall levels (17%) of PPNL found in this study when considering the whole breeding herd (n=870) are characteristic of the degree of PPNL found in other herds under scrutiny. Nevertheless, when only first service goats (n= 533) were studied in an effort to determine the effect of hormonal synchronization, PPNL was greater than that

observed previously. This result (see next subheading) is clear indication of the negative effects of hormonal protocols for E/OS since only first time breeders were synchronized.

Comparison of the extent and distribution of early progeny wastage occurrence between groups analyzed

The prenatal losses encountered across all variables considered in this study were evaluated on the basis of: a) all goats bred, regardless of times bred, (n=870) and, b) goats that were bred for the first time (n=533).

Overall (all breedings) group

Assuming no fertilization failure there was 72% CR with a PPNL of 28%. The time distribution of the sustained losses was 64% occurring early and 36% happening late. More specifically of all the losses accrued there was: 64% embryonic mortality, 19% stillborns, 11.3% abortions, 2% of the progeny died because the dams underwent C-sections and 1.3% of the newborn died within 24 h of birth.

First breeding (mean effects) group

Again, assuming no fertilization failure, results germane to only the 1st breeding group of females, the CR was 67% which means a PPNL of 33% was sustained. Although not statistically independent groups, there was a difference between the CR attained for the overall group and that obtained for the mean effects group ($P<0.014$). The time distribution of the losses was 62% early losses and 38% late losses. The PPNL breakdown in loss categories turned out to be the same for both overall and mean effects group ($P>0.864$) with a 62% of the losses occurring as EM, 23% in the form of stillborns, 12% abortions and 1% of does receiving a C-section with progeny death

This estimated results of the magnitude, but not the time of occurrence, agree in general with the results found for different temperate breeds of sheep where most of the early pregnancy losses are believed to occur as early embryonic death at an estimated rate between 6% and 48% of all zygotes.[75, 76]

However, in the bovine, it has been calculated that the embryonic and fetal mortality rate (excluding fertilization failure) is 56%. and 40% for a high-producing and moderate-producing dairy cows respectively, with an estimated 70–80% of the losses sustained between 8 and 16% dPB.[43] In other studies it has been documented that the variation of early pregnancy loss in pasture-based production of dairy cows can be as much as 22% with a much higher mortality proportion of embryos dyeing 7 dPB (insemination) in contrast to heifers and lower yielding cows.[77]

However, many of the results arrived at, in this study, are not mutually consistent, conclusions arrived at depend on whether or not we analyze the whole breeding herd with all the breeding or only first breeding goats. The timing of losses due to specific effects can sway the inferences as well and promote antagonistic conclusions. For example, in this study, early progeny wastage occurred in all 6 breeds considered during the two breeding seasons studied (n=870) but the distribution of losses among breeds was different. EM contributed from 50% (Boer) to 91% (Angora) of the total PPNL within breed losses and from 15% (in Spanish) to 27% (Boer) in the case of SB.
If in fact losses do occur, more than previously thought, late in development, as in the example provided, this would agree with preliminary data collected by the group of Aimee Wurst and Charlotte Clifford-Rathert and colleagues where according to the authors of one of the studies published[78] the results suggests that caprine losses are

evidenced at different rate rather than as those found in sheep. Moreover, that losses in goats would be occurring primarily relatively late in pregnancy. It is important to point out that this previous work considers exclusively herds that used natural service and no ART's were involved.

Factors which influence early progeny wastage in goats

Prenatal and perinatal goat losses were influenced by most of the independent classification variables studied (i.e., breed, age, parity, P4 exposure, breeding procedure, number of breedings, and the interaction between PG600 and Parity) as well as the associated variable of litter size. Variables that did not affect PPNL were PG600, breeding/kidding season and AI technician (in the groups where AI was conducted). From the results obtained with first-time bred goats it can be inferred that the E/OS protocol used does not influence negatively early progeny wastage in goats. We can surmise that much of the early losses are potentially connected to stress which trigger premature CL regression before implantation has taken place. Although the application of E/OS protocols may not alter the chemical environment conducive to conception it may very well be that the artificial breeding procedures used are themselves stressors of undefined and not quantified influence. Particularly hormonal response to vaginal speculum entry and excessive manipulation in attempts to traverse the cervix may be implicated in triggering neural responses leading to the release of oxytocin, prosta-glandin and/or epinephrine which could be implicated in modifying CL dynamics. It is not difficult to envision that changes in the hormonal milieu can affect considerably the conceptus and the uterine environment in what is itself by this time not even a "chemical pregnancy" since maternal recognition, nor embryo attachment have taken place yet.

Agreement Analysis

Comparisons made between levels of blood plasma P4 at 22 and at 42 dPB (see figure 12) shows that data on the diagonal of the plot is quite dispersed on either side of the line of equality, visually suggesting lack of agreement between the hormone levels. This is to be expected if there is early embryonic loss –whether partial or total- between the two time points.

The above results are further corroborated by The ANOVA to which the data was applied supports this conclusion since it does indicate that the means of both timed samples were different ($P<0.02$) and by the fact that when Cohen's (κ) was used to determine the level of agreement between P4 blood plasma levels at 21 and at 42 dPB the number of calculated embryos was different ($P<0.01$) between 21 dPB blood plasma P4 and 42 dPB, between 21 dPB collected blood plasma P4 and 42 dPB UI, and between 21 dPB blood plasma P4 and litter size at kidding. Whereas using Bowker's test the conclusion is that both time points of blood plasma collection do not satisfy symmetry.

Limitations and future directions

Fertilization rate

This and other similar studies have the limitation that fertilization rate was not established. Therefore, the extent of PPNL includes fertilization failure. Since very early research on reproductive wastage in small ruminants (Restall et al, 1978) it has been recognized that losses due to fertilization failure and early embryonic mortality are difficult to distinguish from one another and, to date, have not been assessed under field

conditions. This problem is of particular interest in litter bearing species where there is need to establish between fertilization failure and total or partial embryonic death.[41]

Partial embryonic loss

In this study partial loss, when more than one embryo was conceived, was not taken into account separately but was accounted in the final results of losses. Due to the complexity of events associated with fertilization: embryo genome and epigenome activation, embryo development, maternal recognition of pregnancy, embryo attachment, placentation and *corpus luteum* (CL) maintenance, embryonic losses early in pregnancy are usually much higher than fetal losses at later stages of gestation, and can be as high a 20-30%,[79]. In cattle and sheep embryonic and fetal deaths during pregnancy account for 25 to 50% of the total number of fertilized ova.[80, 81]

In general, losses due to early embryonic mortality are difficult to assess under field condition[82, 83] and actual embryonic death, for cases of multiple ovulation and fertilization, consequently, mortality is partial (i.e., only one embryo survives from a batch of two or more embryos), and is largely unreported,[84] effectively introducing a downward bias to EM estimates and, therefore, PNL. Moreover, as has been discussed by [85] assessments of EM fail to distinguish between fertilization failure and actual embryonic death. The blood plasma hormonal levels P4 and E_2 may lend themselves to scrutiny to determine if losses were partial or total.

Premature CL regression

Premature CL: regression was evaluated only from a behavioral standpoint (i.e., a doe coming back to estrus 5 to 10 dPB). Endocrine confirmation of such an event could have been performed by blood plasma P4 analysis.

Pre-attachment embryo loss

Losses that take place before the embryo attaches to the uterine wall were not evaluated with the degree of rigor required. Losses accrued while the conceptus is in the embryonic stage are difficult to determine specially those related with events prior to maternal recognition of pregnancy (day 15-16) and/or dealing with the pre-implantation embryo (18 d), and before the first pregnancy diagnosis (20 to 22 dPB) can be performed. This study made no attempt to determine pre-attachment and pre-maternal recognition of pregnancy losses but did consider embryonic mortality from putative maternal recognition of pregnancy until the embryo/fetus developmental transition (around day 40-42).

To this effect, embryo mortality was based on two biological hypotheses formulated by Geisler (1977)[57] and adapted in this study as follows: **a)** The *all-or-nothing fertilization hypothesis*, which states that a female that is naturally serviced or is AI'ed will end up with either all or none of her released ova fertilized, and **b)** The *survival/demise independence hypothesis* for embryo persistence/death, which states that the survival of a fertilized ovum depends only on how many ova were released with it, and is independent of the survival or death of other oocytes ovulated at the same time.

Conclusion

This study is the first in the U.S. to show the high incidence of early progeny wastage and when these losses occur during gestation of goats managed both extensively and intensively within a scenario of productive trait genetic improvement by means of assisted reproductive technologies using commercially obtained semen and breeding by both fixed time and non-fixed time.

This study revealed the extent of early losses that drive a low kidding rate of 49%. It has also been demonstrated that across breeds, age and parities conception rate is relatively high (72%) considering the negative effect of transcervical insemination coupled to fixed-time breeding has as documented in this study. Most of the losses occur early although the amount of stillbirths requires considered research attention. By the time 45-d ultrasound imaging diagnosis for pregnancy occurs the survival rate is only 60%.

There are several results of concern deserving further closer scrutiny. For example, there was documented evidence of considerable stillbirths in the alpine breed without a logical explanation for the finding, goats bred by TrAI were almost 14× more likely of having early losses than goats bred by NS. Since the greatest number of embryos/fetuses lost was attributed to primiparous goats at 47% this may necessitate a breeding program with greater attention provided to this parity category. Likewise, result were not consistent for all breeds or age categories. Early progeny wastage as well as the associated litter size variable, were influenced by at least one of the treatments considered. That is, breed, age, parity, P4 exposure, breeding procedure, number of breedings, and the interaction between PG600 and parity. Although PG600 influenced early prenatal loss when given at low (1.75 mL) dosage no other effects on reproductive performance were detected.

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Table 1. Nominal logistic model [1] fit for goat for 2-year pregnancy data.

Whole Model Test [1]

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	79.55407	29	159.1081	<0.0001*
Full	369.31244			
Reduced	448.86651			

RSquare (U): 0.1772 **Observations (or Sum weights):** 666

Lack of fit (LOF)

Source	DF	-LogLikelihood	ChiSquare	Prob>ChiSq
Lack of fit	198	126.00987	252.0197	0.0056*
Saturated	227	243.30257		
Fitted	29	369.31244		

Effect Likelihood Ratio Tests

Source	Nparm	DF	L-R ChiSquare	Prob>ChiSq
Breed	5	5	30.1247163	<0.0001*
Age group	3	3	18.8907236	0.0003*
Parity	2	2	7.07473025	0.0291*
Breeding procedure	1	1	4.81805997	0.0282*
Breeding number	2	2	5.86792329	0.0532
P4 exposure	4	4	32.1663214	<0.0001*
PG600	2	2	0.4154449	0.8124
Age group × PG600	6	6	11.998437	0.0620
Parity × PG600	4	4	6.38037123	0.1725

Table 2. Prenatal/perinatal loss rates and 95% confidence intervals.

[Panel A]

Cause	Count	Rate	Lower 95%	Upper 95%
Embryo mortality	96	0.640	0.518	0.782
Stillborn	29	0.193	0.130	0.278
Aborted	17	0.113	0.066	0.182
C-Section	3	0.020	0.004	0.058
Pregnant doe death	3	0.020	0.004	0.058
Perinatal loss	2	0.013	0.002	0.048
Dystocia	---	---	---	---
Pooled Total	150	0.167	0.141	0.196

[Panel B]

Cause	Count	Rate	Lower 95%	Upper 95%
Embryo mortality	96	0.640	0.518	0.782
Stillborn	29	0.193	0.130	0.278
Aborted	17	0.113	0.066	0.182
C + MD + PNL	8	0.053	0.023	0.105
Pooled Total	150	0.250	0.212	0.293

Table 3. Total number of embryos produced over the course of the study (n=879).

Breed	Number of goats with embryo groups of:					Goats not UI scanned	Total number of embryos/ goats	Prolificacy
	None	Single	Twins	Triplets[a]	Fluid vesicle			
Alpine	42	30	39	7	2	21	131/99	1.32
Angora	20	4	11			57	26/72	0.36
Boer	57	31	55	3	1	22	151/112	0.89
Spanish	2	19	18	5		17	70/61	1.15
Stiff-leg	35	4	10		1	20	25/70	1.35
X-Breeds	119	51	99	4	5	68	266/227	1.17
Total	**275**	**139**	**232**	**19**	**9**	**205**	**669/604**	**1.11**

[a]Includes 7 litters of quadruplets and 1 litter of quintuples.

Table 4. Number of progeny and pre/peri-natal loss components by goat breed.

Breed	N° of progeny		Pre/Peri-natal loss components (PPNL)								
			A[a]	C[b]	MD[c]	EM[d]		PPL[e]	SB[f]	Total	
	Total	Survive				N°	%			N°	%
Alpine	129	108	1	3	2	21	16		9	36	28
Angora	26	16				10	38	1		11	42
Boer	150	137	6			13	9		7	26	17
Spanish	70	59				11	16		2	13	19
T. Stiff-Leg	24	14	2			10	42			12	50
x-Breeds	261	230	8		1	31	12	1	11	52	20
Grand Total	**660**	**564**	**17**	**3**	**3**	**96**	**15**	**2**	**29**	**150**	**23**
			11%	2%	2%	64%	100%	1%	19%		

[a]Abortion, [b]Cesarean section, [c]Mother's death, [d]Embryo mortality, [e]Postpartum loss, [f]Stillborn.

Table 5. Odds ratios (OR) for main treatment effects,[a]

Goat breed					
				95% Confidence interval	
Level1	**Level2**	**Odds Ratio**	**Prob>Chisq**	**Lower**	**Upper**
Spanish	Alpine	222.36702	<.0001*	11.112108	13285.999
Spanish	Boer	77.492074	0.0005*	5.9030904	2444.1458
Stiff-Leg	Spanish	0.0249137	0.0442*	0.0003817	0.9104174
X-breds	Alpine	13.759368	0.0097*	1.8008171	188.18655
X-breds	Boer	4.7949645	0.0394*	1.0769203	25.22815
X-breds	Spanish	0.0618768	0.0154*	0.0024015	0.611118
P4 exposure in E/OS synchronization protocol					
				95% Confidence interval	
Level1	**Level2**	**Odds Ratio**	**Prob>Chisq**	**Lower**	**Upper**
NNT	N	16.77122	0.0383*	1.1541526	388.15495
PG600 dosage level used in the E/OS synchronization protocol					
				95% Confidence interval	
Level1	**Level2**	**Odds Ratio**	**Prob>Chisq**	**Lower**	**Upper**
5	1.75	6.8438632	0.0535	0.9707841	69.747951
Breeding procedure					
				95% Confidence interval	
Level1	**Level2**	**Odds Ratio**	**Prob>Chisq**	**Lower**	**Upper**
NSp	LAI	0.0968997	0.0156*	0.0088748	0.6598861
Tr-AI	NSp	13.647647	0.0311*	1.2425072	301.36951
Parity category					
				95% Confidence interval	
Level1	**Level2**	**Odds Ratio**	**Prob>Chisq**	**Lower**	**Upper**
Nullipar.	Multipar.	12.24416	0.0036*	2.1752431	92.987406

[a]Only statistically significant and stably calculated OR's are included.

Table 6. Early goat progeny wastage and conception (adjusted for fertile females based on prenatal loss) as a function of breeding procedure and PG600 dose.

1st breeding	LAI	NSp	TrAI	Total	
PG600 (0.0 mL)				**231**	
Open	11	52	16	79	
Pregnant	15	94	6	115	
SB	---	11	2	13	**PPNL=37;16%**
Aborted	1	3	1	5	
EM	3	13	3	19	
					Conceived=152; 66%
PG600 (1.75 mL)				**196**	
Open	22	43	23	88	
Pregnant	10	49	5	64	
SB	2	5	---	7	**PPNL=44; 22%**
Aborted	3	3	---	6	
EM	12	12	6	30	
MD	---	1	---	1	
					Conceived=108; 55%
PG600 (5.0 mL)				**106**	
Open	17	19	6	42	
Pregnant	4	35	5	44	
SB	1	2	---	3	**PPNL=20; 19%**
Aborted	---	1	---	1	
EM	5	5	4	14	
C	1	---	---	1	
MD	---	1	---	1	
					Conceived=64; 60%
Grand Total	107	349	77	**533**	
Total open	50	114	45	209 (39%)	
Total pregnant	29	108	16	223 (42%)	
Total PPNL	28	57	16	101 (19%)	
Total conception	57	165	32	324 (61%)	

Table 7. Does bred and embryos number by breeding procedure and PG600 dose.

	Litter Size	N° of does			Does:		N° of progeny			Subtotal
		LAI	NSp	TrAI	Bred	conceived	LAI	NSp	TrAI	Progeny
PG600= 0.0 mL	*total* ➤	30	173	28	231					
	0	11	52	16	79					
	1	6	37	9	52		6	37	9	
	2	9	74	2	85	152	18	148	4	
	3	4	9	1	14		12	27	3	
	4		1		1		0	4	0	268
PG600= 1.75 mL	*total* ➤	49	113	34	196					
	0	22	43	23	88					
	1	13	29	6	48		13	29	6	
	2	11	24	4	39		22	48	8	
	3	2	13		15	108	6	39	0	
	4	1	3	1	5		4	12	4	191
	5		1		1					
PG600= 5.0 mL	*total* ➤	28	63	15	106					
	0	17	19	6	42					
	1	6	10	6	22		6	10	6	
	2	4	27	2	33	64	8	54	4	
	3	1	6	1	8		3	18	3	
	4		1		1		0	4	0	116
Total does bred ➤		107	349	77	**533**	324	Total progeny=			**575**

Table 8. Half matrix for Cohen's agreement (*κ*) and Bowker's symmetry measures (probabilities of rejection of the null hypothesis; *Ho*).

	Reproductive status determined by:			
	21d P4	**42d P4**	**45 d UI**	**Kidding**
Non Return *(κ)*[a]	0.2216	0.0190	0.0001	0.0001
***Bowker's*[b]**	0.0782	0.0396	0.2998	0.2059
21d P4 *(κ)*	---	0.1584	0.3172	0.4825
Bowker's	---	0.8694	0.0325	0.2743
42d P4 *(κ)*	---	---	0.0504	0.0261
Bowker's	---	---	0.0104	0.1701
45d UI *(κ)*	---	---	---	0.0001
Bowker's	---	---	---	0.0587

[a]*Ho:* The value of $\kappa = 0$; i.e., there is no agreement among raters.
[b]*Ho:* The probabilities satisfy symmetry.

Table 9. Half matrix of probabilities of Cohen's (*κ*) and Bowker's agreement measures for progeny number predictors.

	Progeny number determined by:		
	42d P4	**45 d UI**	**Litter size**
21d P4 ***(κ)***[a]	0.0092	0.00008	0.0060
Bowker's[b]	0.0790	0.0005	0.0001
42d P4 ***(κ)***	---	0.0004	0.0018
Bowker's	---	0.5729	0.0781
45d UI ***(κ)***	---	---	0.0001
Bowker's	---	---	0.0675

[a]*Ho:* The value of $\kappa = 0$; i.e., there is no agreement among raters.
[b]*Ho:* The probabilities satisfy symmetry.

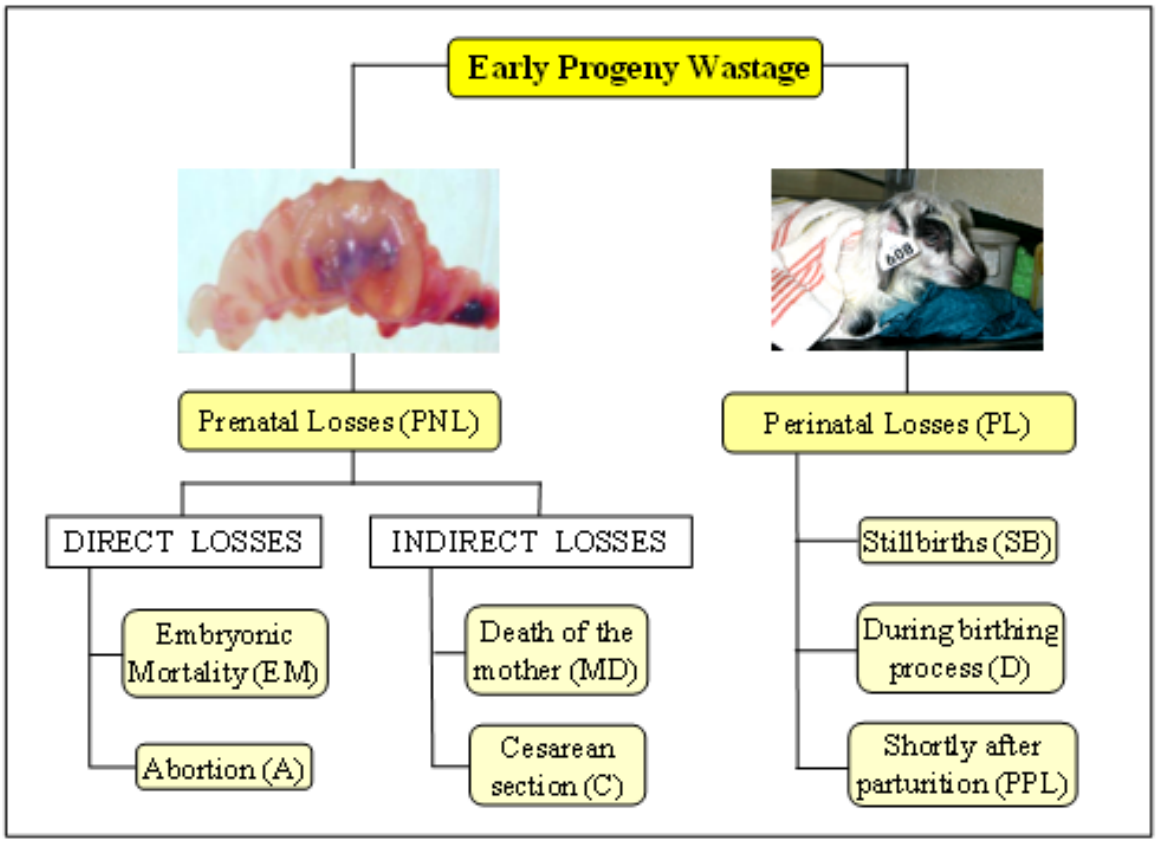

Early Progeny Wastage
Prenatal Losses (PNL)
Perinatal Losses (PL)
DIRECT LOSSES
INDIRECT LOSSES
Embryonic Mortality (EM)
Abortion (A)
Death of the mother (MD)
Cesarean section (C)
Stillbirths (SB)
During birthing process (D)
Shortly after parturition (PPL)

Figure 1. Mortality components of early progeny wastage in goats.

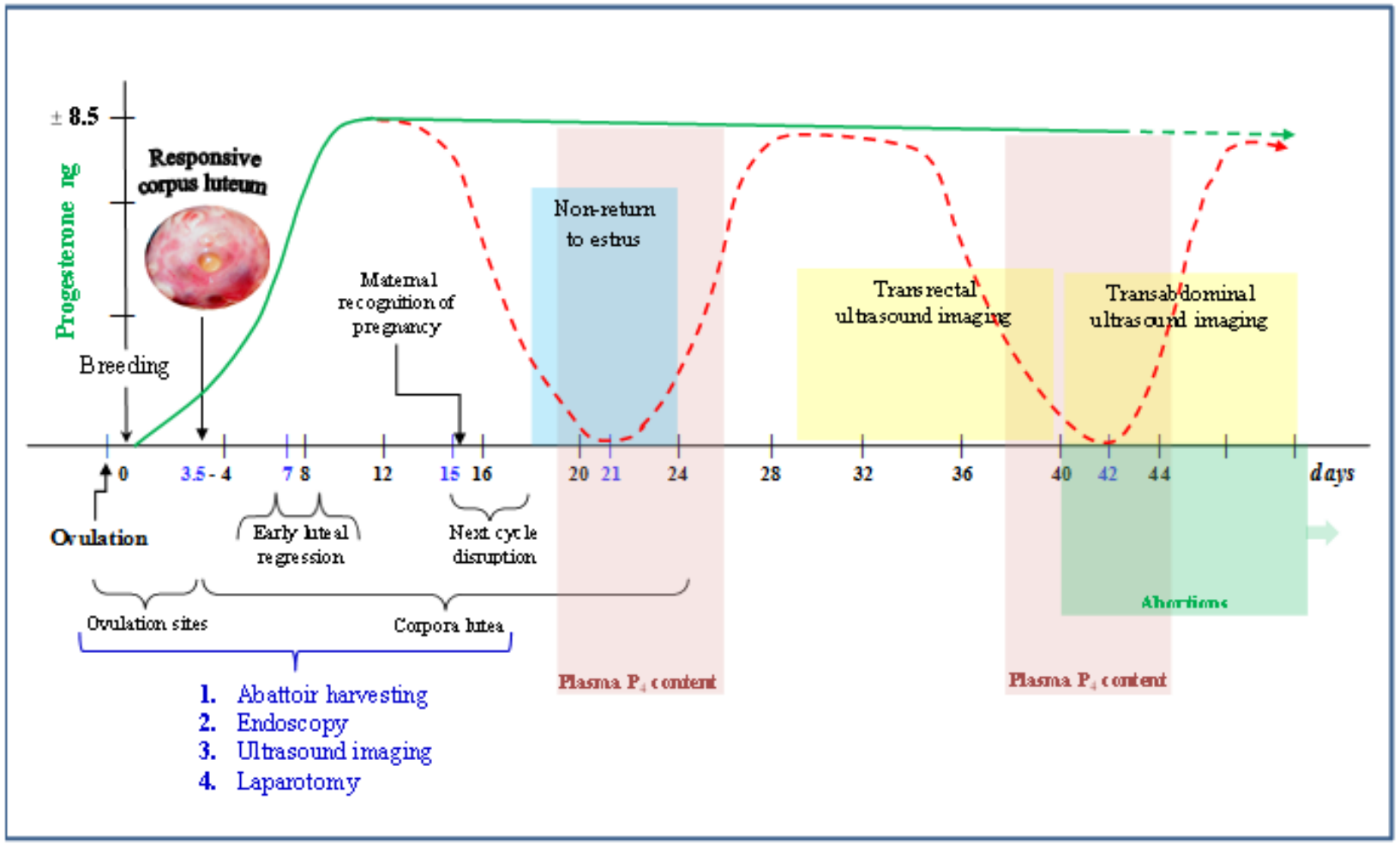

± 8.5
Progesterone ng
Responsive corpus luteum
Breeding
Maternal recognition of pregnancy
Non-return to estrus
Transrectal ultrasound imaging
Transabdominal ultrasound imaging
0
3.5 - 4
7 8
12
15 16
20 21
24
28
32
36
40
42
44
days
Ovulation
Early luteal regression
Next cycle disruption
Ovulation sites
Corpora lutea
Abortions
Plasma P4 content
Plasma P4 content
1. Abattoir harvesting
2. Endoscopy
3. Ultrasound imaging
4. Laparotomy

Figure 2. The use of corpus luteum CL dynamics and pregnancy diagnosis throughout gestation for early detection of goat progeny wastage. The abscissa in the graph depict only the first 48 days of a 150 gestation period. The Y ordinate axis is an approximate scale of usual progesterone (P4) levels found through the goat estrous cycle and gestation period. Ovulation occurs before breeding and the CL becomes responsive around day 3.5 post ovulation. P4 levels increase from about 1 ng for non-pregnant goats to 8-12 ng for pregnant goats or goats in the luteal phase of the estrous cycle. Upon early luteal regression, usually occurs between day 6 to 9, females show spontaneous estrus shortly thereafter P4 values remain high if fertilization occurs and there is maternal recognition of pregnancy on day 15-16. Pregnancy can be diagnosed by non-return to estrus on days 18 through 24 [blue shading], from day 19 to 25 by blood plasma P4 using radioimmunoassay (RIA) or an enzyme linked immunosorbent assay (ELISA) [Pink shading], transrectal ultrasound imaging (UI) as early as29 days post-breeding (dPB) [yellow shading], and transabdominal UI as early as 40 dPB. Late losses can be visually detected after day 40 of gestation [green shading]. Early losses can be detected by: 1. Abattoir harvesting, endoscopy of ovulation sites, UI of ovulation or CL dynamics, laparotomy of reproductive tract, or by atypical estrous cycle length.

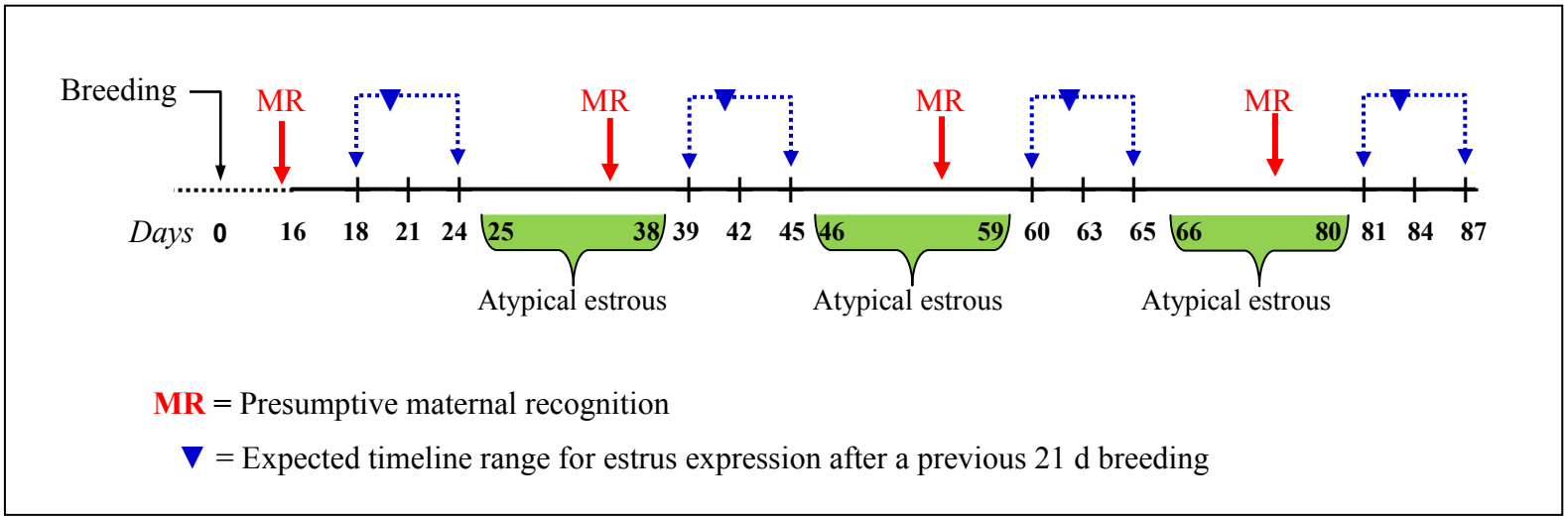

Breeding
MR
MR
MR
MR
Days 0 16 18 21 24 25 38 39 42 45 46 59 60 63 65 66 80 81 84 87
Atypical estrous
Atypical estrous
Atypical estrous
MR = Presumptive maternal recognition
▼ = Expected timeline range for estrus expression after a previous 21 d breeding

Figure 3. Atypical estrous intervals used to describe estimated conception in bred goats. It was considered that conception (i.e., embryo attachment) had occurred if at least one embryo attached to the uterus under the premise that there was no return to estrus by day 17 up to day 25 PB or if there was a return to estrus, after having been bred, in an atypical estrous cycle period of days (*i.e.,* > 24 to <39 for first cycle post breeding and > 24 to <39, > 45 to < 60 d and >66 < 81 for second cycle post breeding).

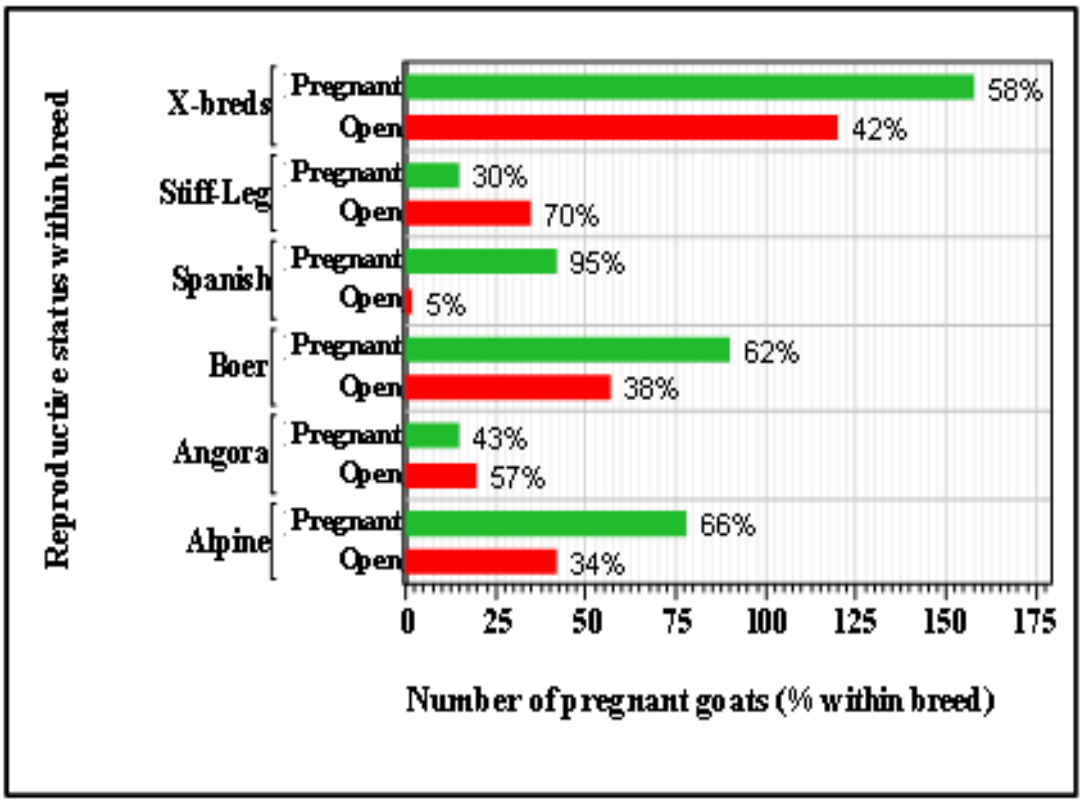

Reproductive status within breed
X-breds
Pregnant
Open
58%
42%
Stiff-Leg
Pregnant
Open
30%
70%
Spanish
Pregnant
Open
95%
5%
Boer
Pregnant
Open
62%
38%
Angora
Pregnant
Open
43%
57%
Alpine
Pregnant
Open
66%
34%
0
25
50
75
100
125
150
175
Number of pregnant goats (% within breed)

Figure 4. Global pregnancy rate (n= 666) according to goat breed for two breeding seasons. Green histogram bars represent goats determined to be pregnant by 45 d post-breeding transabdominal ultrasound imaging. Red histogram bars represent goats determined to be non-pregnant (open).

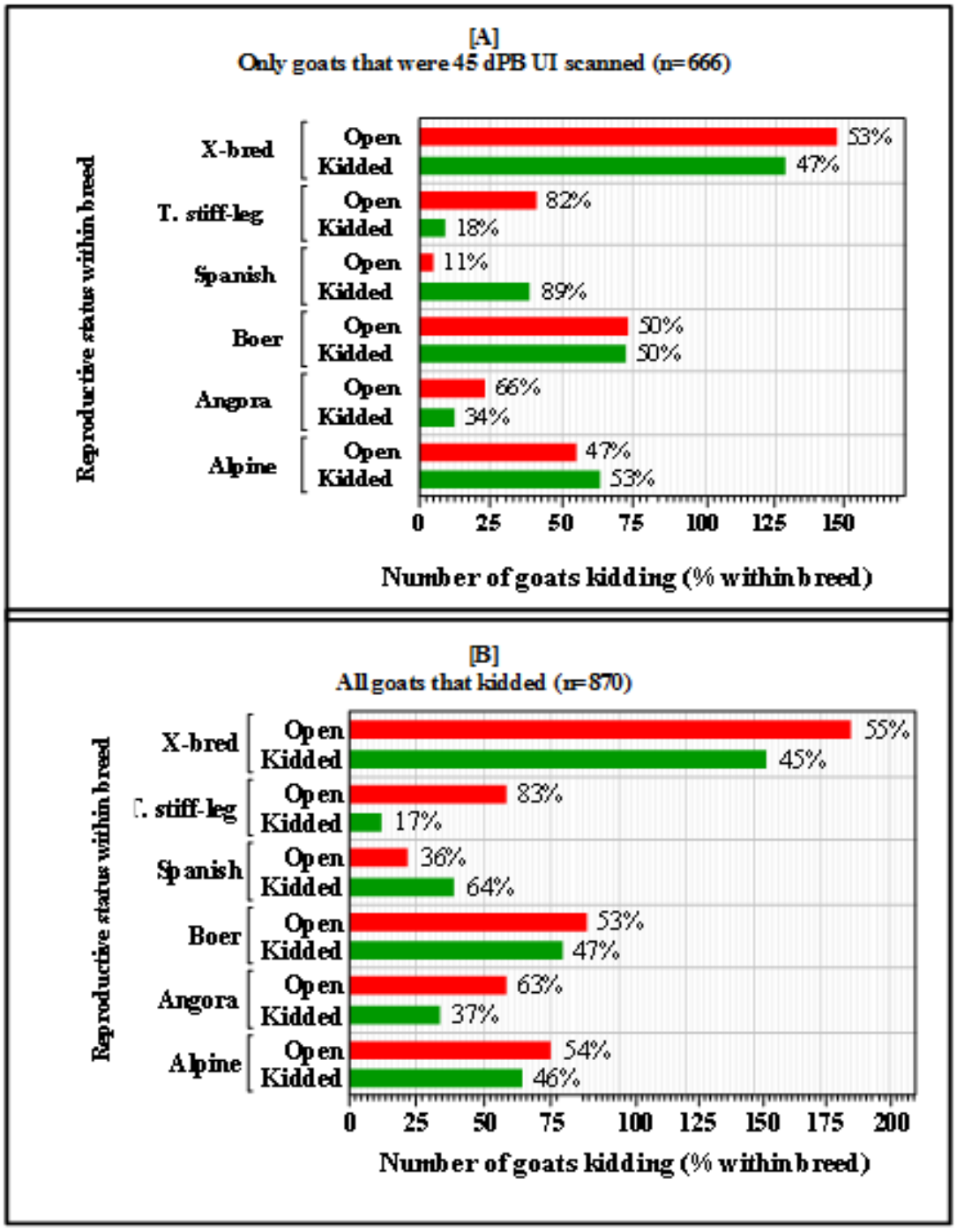
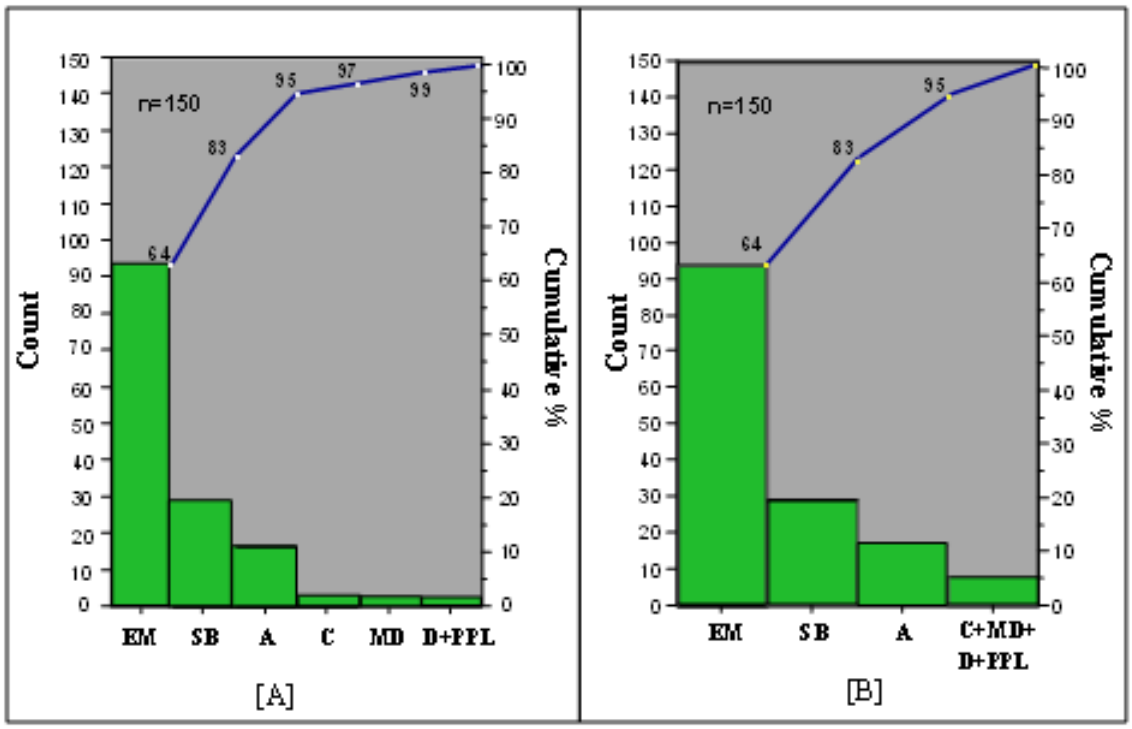

[A]
Only goats that were 45 dPB UI scanned (n=666)
Reproductive status within breed
X-bred
T. stiff-leg
Spanish
Boer
Angora
Alpine
Open
Kidded
53%
47%
82%
18%
11%
89%
50%
50%
66%
34%
47%
53%
0
25
50
75
100
125
150
Number of goats kidding (% within breed)
[B]
All goats that kidded (n=870)
Reproductive status within breed
X-bred
T. stiff-leg
Spanish
Boer
Angora
Alpine
Open
Kidded
55%
45%
83%
17%
36%
64%
53%
47%
63%
37%
54%
46%
0
25
50
75
100
125
150
175
200
Number of goats kidding (% within breed)

Figure 5. Parturition status of goats by breed for two kidding seasons. Panel [A} only goats that were 45 d post-breeding UI scanned (n=666). Panel [B] all goats that kidded (n=8700. Green histogram bars represent goats That kidded and red histogram bars represent goats that did not kid (open).



[a]Panel [A] has all six PPNL categories. In panel [B] the last three categories of panel [A] were combined to make one category.

[b]EM: Embryonic mortality, SB: Stillbirth, A: Abortion, C: Cesarean section, MD: mother's death, D: Dystocia, and PPL: Postnatal loss.

Figure 6. Pareto plot of goat prenatal and perinatal loss components from two breeding/kidding seasons. Panel [A] has all six pre/peri-natal (PPNL) categories: embryonic mortality (EM), stillbirth (SB), abortion (A), cesarean section (C), maternal death (MD), dystocia (D), and post-partum losses (PPL). In panel [B] the last four categories of panel [A] were combined to make only one third category.

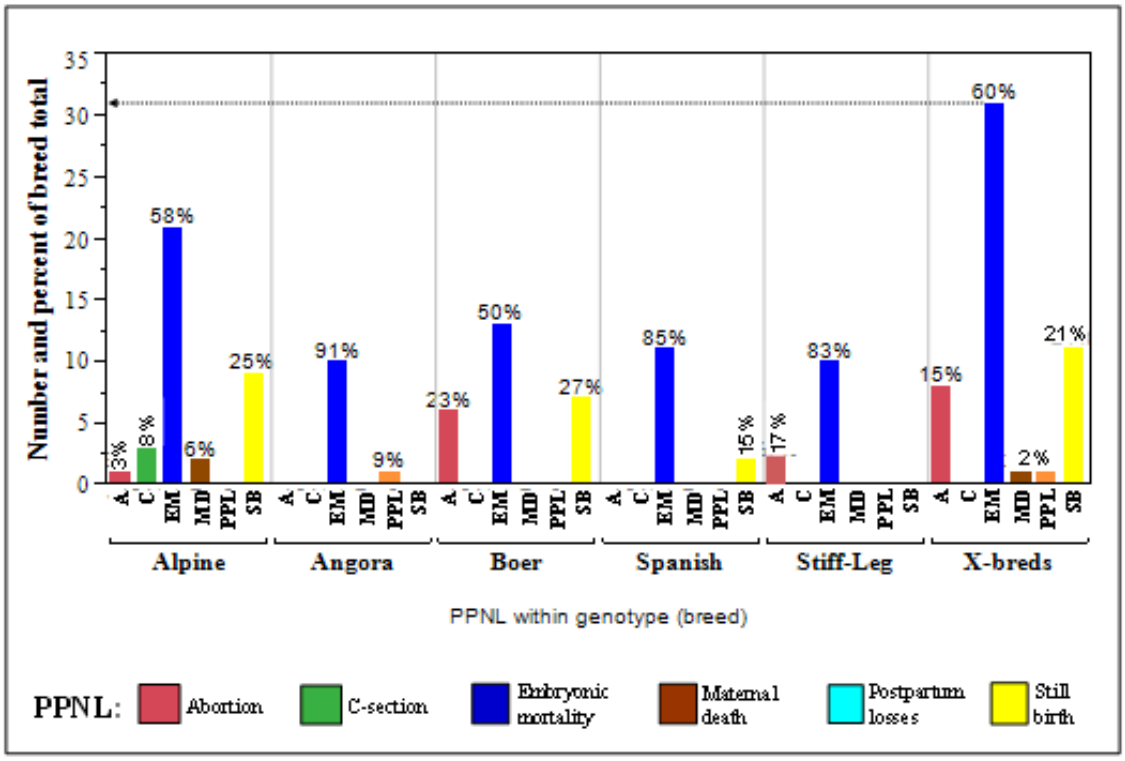

Number and percent of breed total
35
30
25
20
15
10
5
0
3%
8%
58%
6%
25%
91%
9%
23%
50%
27%
85%
15%
17%
83%
15%
60%
2%
21%
A
C
EM
MD
PPL
SB
Alpine
Angora
Boer
Spanish
Stiff-Leg
X-breds
PPNL within genotype (breed)
PPNL:
Abortion
C-section
Embryonic mortality
Maternal death
Postpartum losses
Still birth

Figure 7. Prenatal and perinatal progeny loss (PPNL) incidence (%) as a function of goat breed. Global early progeny loss is given both as the total number of observations in the ordinate axis, which coincides with the top of each category in the bar graph, and also as a percentage of total losses per breed in which case the percent number is placed on top of each of the bar graph category columns.

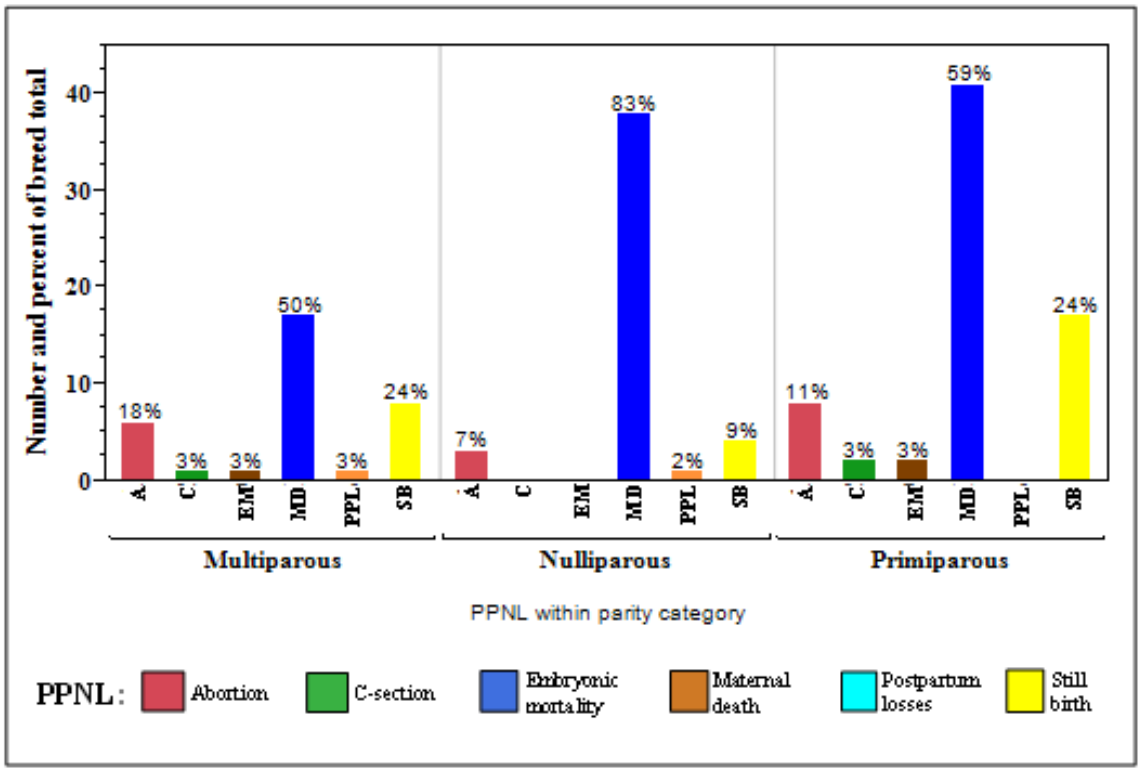

Number and percent of breed total
40
30
20
10
0
18%
3%
3%
50%
3%
24%
7%
83%
2%
9%
11%
3%
3%
59%
24%
A
C
EM
MD
PPL
SB
Multiparous
Nulliparous
Primiparous
PPNL within parity category
PPNL:
Abortion
C-section
Embryonic mortality
Maternal death
Postpartum losses
Still birth

Figure 8. Prenatal and perinatal progeny loss (PPNL) incidence (%) as a function of goat parity category. Global early progeny loss is given both as the total number of observations in the ordinate axis, which coincides with the top of each category in the bar graph, and also as a percentage of total losses per goat parity category in which case the percent number is placed on top of each of the bar graph category columns.

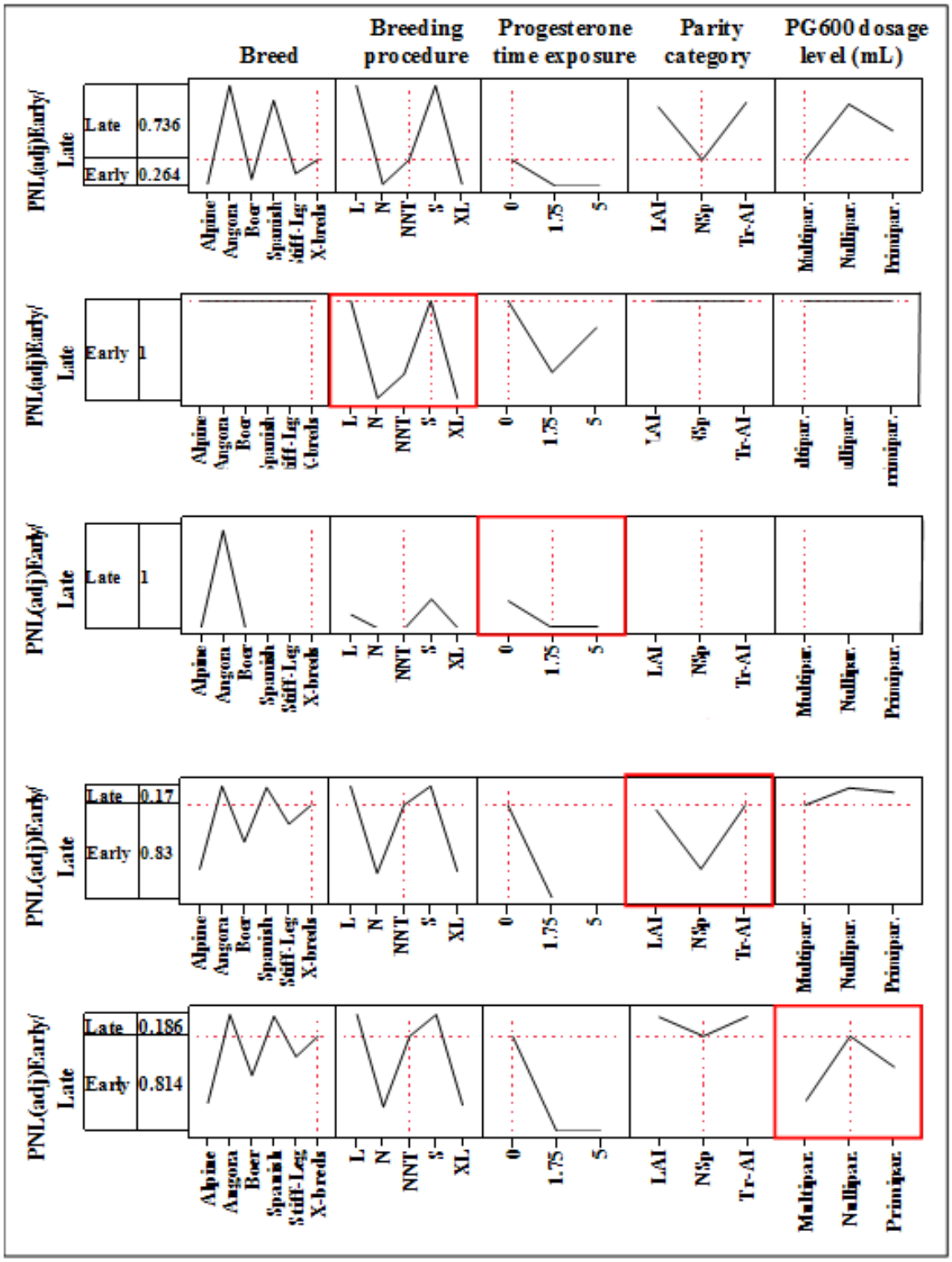

Breed
Breeding procedure
Progesterone time exposure
Parity category
PG600 dosage level (mL)
PNL(adj)Early/Late
Late 0.736
Early 0.264
Early 1
Late 1
Late 0.17
Early 0.83
Late 0.186
Early 0.814
Alpine
Angora
Boer
Spanish
Stiff-Leg
X-breds
L
N
NNT
S
XL
0
1.75
5
LAI
NSp
Tr-AI
Multipar.
Nullipar.
Primipar.

Figure 9. Predicted profiles of early or late goat progeny losses (PPNL) as a function of changes in main treatment effects of first time bred goats (n=533). Predicted profiles were graphed on the basis of statistical logistic regression model [3] (see text).First row panel depicts a typical non-synchronized female. A red lined square in the next panels depicts independent variable change, while the remaining variables are maintained at their original value in relation to the first row. Inside the left column of each row panel the predicted percent early or late PPNL is given in percent progeny loss.

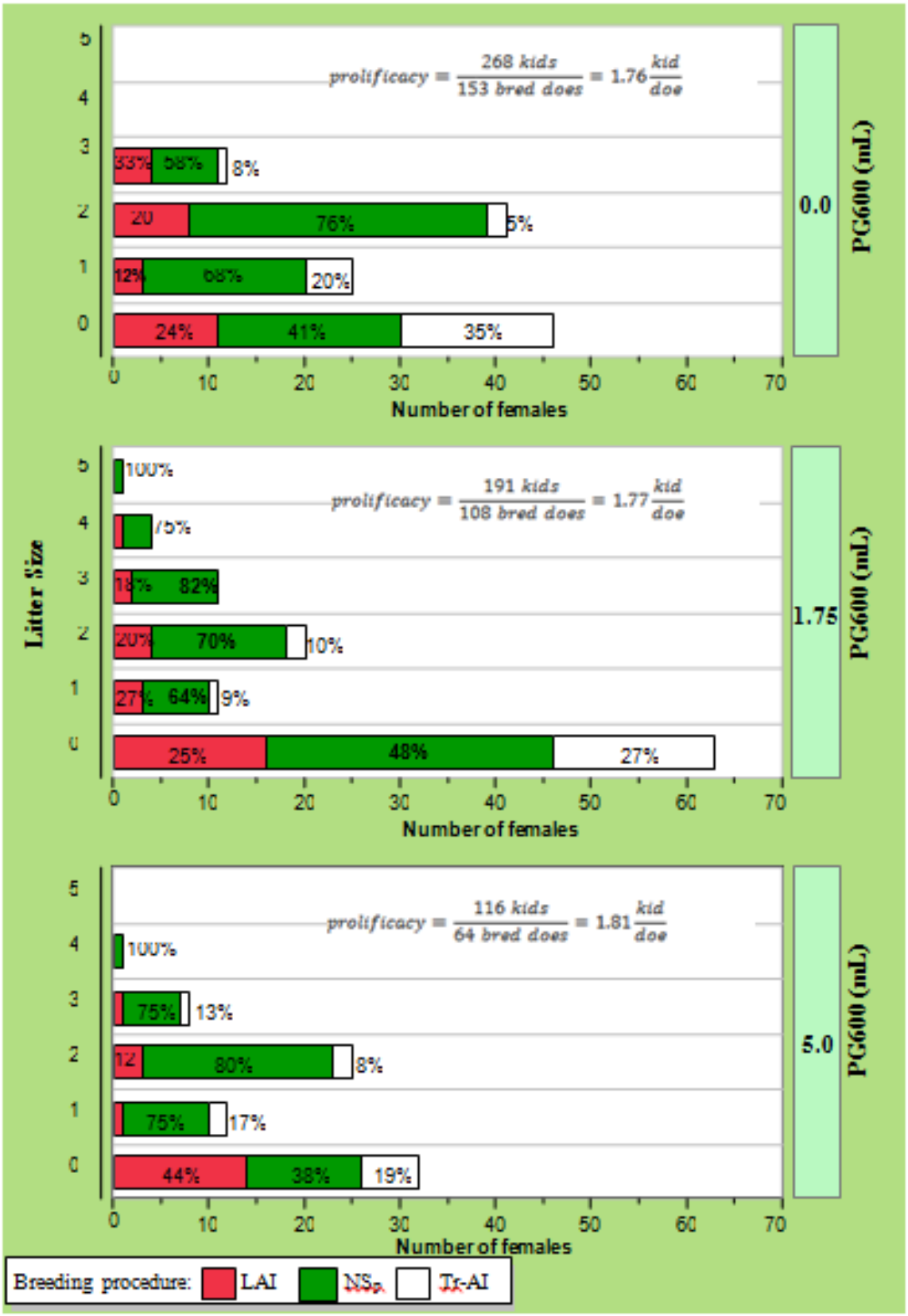

$prolificacy = \frac{268\ kids}{153\ bred\ does} = 1.76 \frac{kid}{doe}$
33%
58%
8%
20
76%
5%
12%
68%
20%
24%
41%
35%
0.0
PG600 (mL)
0
10
20
30
40
50
60
70
Number of females
Litter Size
$prolificacy = \frac{191\ kids}{108\ bred\ does} = 1.77 \frac{kid}{doe}$
100%
75%
18%
82%
20%
70%
10%
27%
64%
9%
25%
48%
27%
1.75
PG600 (mL)
Number of females
$prolificacy = \frac{116\ kids}{64\ bred\ does} = 1.81 \frac{kid}{doe}$
100%
75%
13%
12
80%
8%
75%
17%
44%
38%
19%
5.0
PG600 (mL)
Number of females
Breeding procedure:
LAI
NSp
Tr-AI

Figure 10. Influence of eCG + hCG PG600 within breeding procedure on litter size. Prolificacy is calculated for each panel with the given formula. Red box represents the percent of female goats having a litter size as depicted on the abscissa coordinate (from 0 to 5 kids) as a result of laparoscopy-aided intrauterine (LAI) breeding procedure. Likewise, the green hatched and white boxes portray resulting litter sizes resulting from natural service (NS_p) or transcervical artificial insemination (Tr-AI) breeding procedures, accordingly.

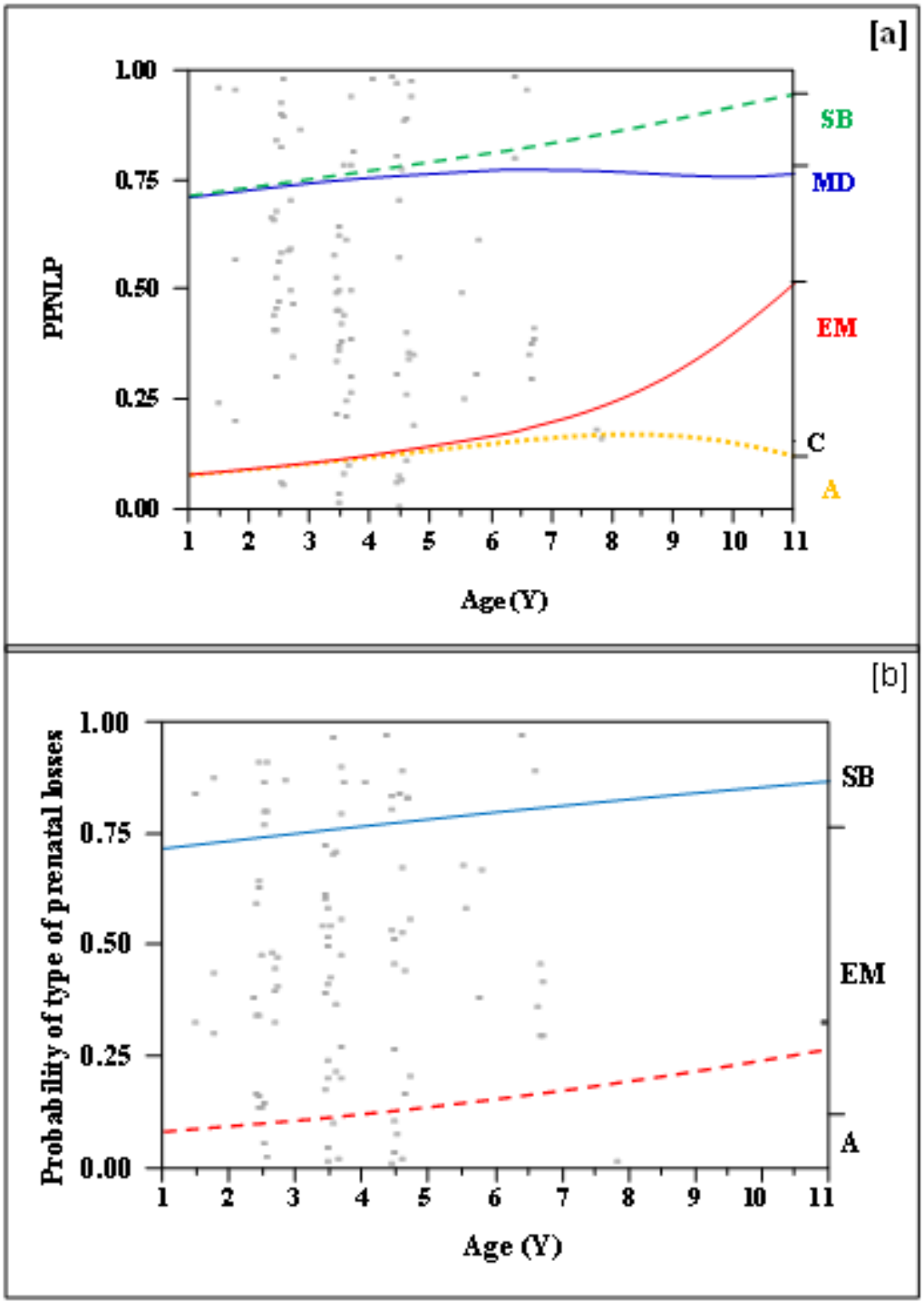

[a]
PPNLP
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
8
9
10
11
Age (Y)
SB
MD
EM
C
A
[b]
Probability of type of prenatal losses
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
8
9
10
11
Age (Y)
SB
EM
A

Figure 11. Scatter graph for a nominal logistic plot: fit for PPNL based on age as a continuous variable. Shown in panel [a] is the effect of age on prenatal and perinatal losses, fitting the data to model [4] (see text). Depicted on panel [b] when the PPNL data was fitted only to the first three early progeny loss categories (i.e., EM, SB, and A). An increase in dam age would result in an increase of the likelihood of a pregnant female experiencing abortion rather than stillbirths. Additionally, an increase in age would mean an increase in the likelihood of an older doe having embryo death compared to stillbirths.

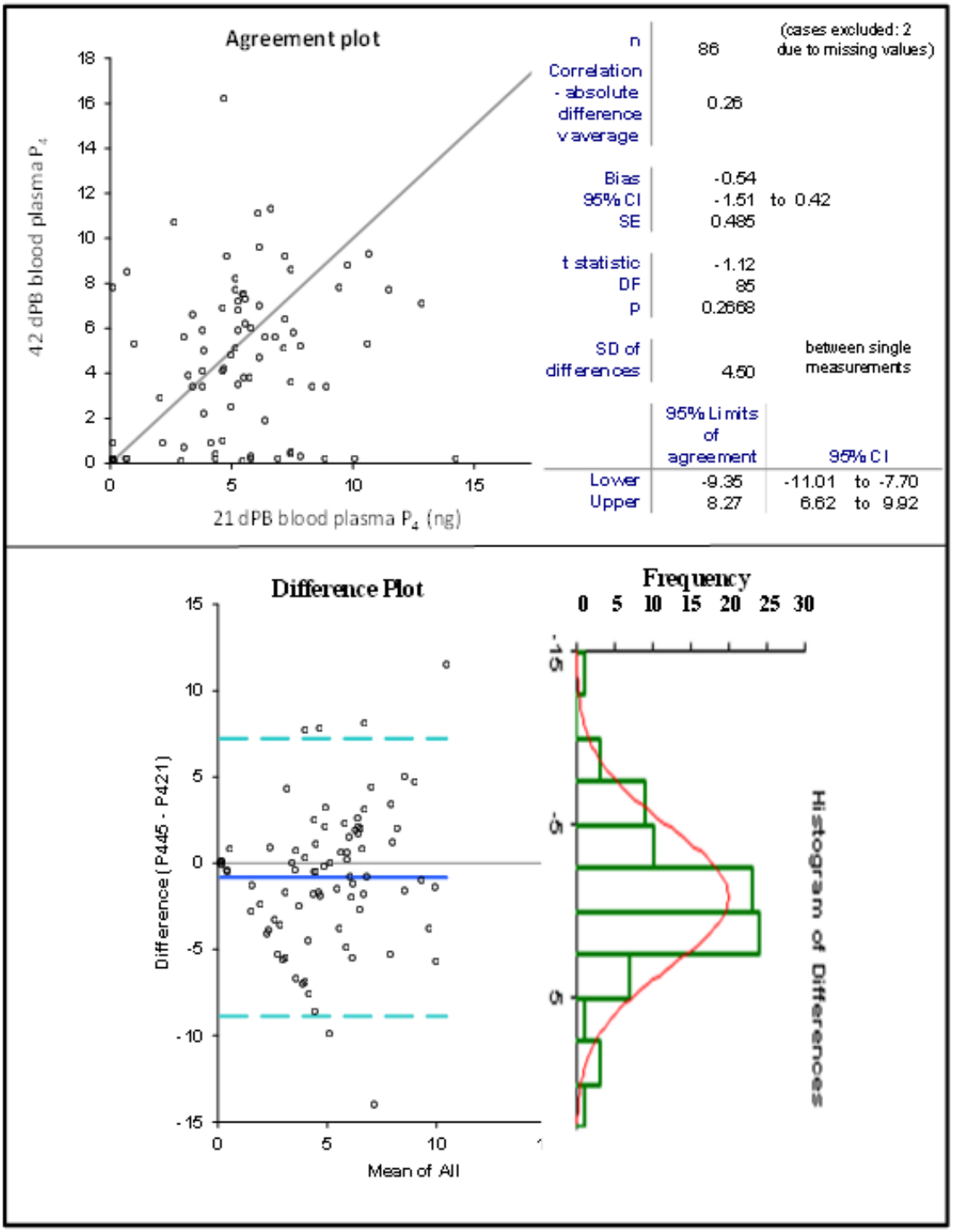

Agreement plot
42 dPB blood plasma P4
21 dPB blood plasma P4 (ng)
0
2
4
6
8
10
12
14
16
18
0
5
10
15
n 86 (cases excluded: 2 due to missing values)
Correlation - absolute difference v average 0.26
Bias -0.54
95% CI -1.51 to 0.42
SE 0.485
t statistic -1.12
DF 85
p 0.2668
SD of differences 4.50 between single measurements
95% Limits of agreement
95% CI
Lower -9.35 -11.01 to -7.70
Upper 8.27 6.62 to 9.92
Difference Plot
Difference (P445 - P421)
Mean of All
15
10
5
0
-5
-10
-15
0
5
10
Frequency
0 5 10 15 20 25 30
Histogram of Differences

Figure 12. Bland-Altman agreement plot for blood plasma progesterone (P4) concentration at 21 and 42 days post breeding. The data on the diagonal presents great dispersion on either side of the diagonal line of equality, suggesting lack of agreement between the hormone concentration of day 21 and 42. The graph calculations show no bias towards the 21 or 42 d data. The difference plot also shows that six of the P4 values were ±2 SD away from the mean difference. Blood plasma P4 presents a normal distribution where almost 50% of the hormone values were within ±5 ng from the mean difference of cero between samples taken on day 21 after breeding compared to samples obtained 42 dPB.

CHAPTER VI

TIME TO TRAVERSE THE CERVIX FOLLOWING VARIABLE TIME PROGESTERONE EXPOSURE AND GONADOTROPIN (ECG AND HCG) LEVELS USING FIXED-TIME TRANSCERVICAL INSEMINATION IN CYCLIC GOATS

Abstract

Effective management in the use of transcervical artificial insemination (TrAI) Effective management in the use of transcervical artificial insemination (TrAI) includes minimizing handling and time to complete procedures. Time to traverse the cervix may be influenced by cervical response following synchronization of estrus and ovulation (E/OS), resulting in disappointing pregnancy rates using TrAI. Uterine cervix response (UCR) to E/OS was evaluated in 213 unselected cyclic Alpine, Boer, Tennessee Stiff-legs and Boer × Spanish crossbred goats. Independent variables were: a) E/OS protocols using CIDR-G as the progesterone (P4) source: extra-long (XL; 24 d), long (L; 12-14 d), short (S; 5-6 d) and a control non-synchronized (NSync) cohort, and b) Use of PG600 at 0.0, 1.75 and 5 cc. Blocking variables were: breed (5), season (’08-’09), age (≤3 y; >3 and ≤4; >4 and ≤5; and >5 y), parity (nulliparous, N; primiparous, P; multiparous, M), breeding procedure (standard TrAI and TrAI-pass with no semen delivered) and AI

technician (2). UCR was evaluated by TrAI mechanical/ technical and biological success rate (SR), TrAI-$SR_{M/T}$ and TrAI-$SR_{B,}$ respectively, and cervical relaxation (depth and time to traverse the cervix).

Data was fitted to ordinal logistic models. Non-significant results were analyzed for practical difference consequence by tests of equivalence. The time to attain TrAI was analyzed by survival analysis techniques using Kaplan-Meir methods and Box proportional hazards regression modeling, risk ratios (RR) were generated for main treatment comparisons. Although TrAI-$SR_{M/T}$ was different among treatments, none of the E/OS protocols influenced TrAI-$SR_{B.}$ Depth of cervix traverse was influenced by: P4 protocol ($P<0.0334$) and parity ($P<0.0052$). Time to penetrate a goat's cervix was influenced by: P4 exposure time ($P<0.0108$), age ($P<0.0268$), parity ($P<0.0028$) and cervical semen placement ($P<0.0001$), while PG600 did not ($P>0.9459$). Goats had a median TrAI time of 3.08 m. Synchronization protocol did influence time to traverse the cervix thereby having a potential effect on success rates of AI in the goat.

Introduction

In general, goat reproduction management has used various hormonal protocols without sufficient validation and results obtained thereof yield conflicting information. Detrimental effects on RP may be a direct result of exogenous synchronization hormones influencing reproductive physiology or indirectly decreasing the likelihood of a normal AI by causing gross anatomical and histomorphological changes of the uterine cervix. Although a complete description of the mechanistic pathway of cervical control during the estrous cycle is still being developed,[1-4] the several factors involved (e.g., anatomical, and/or physiologic)[5-7] are potential candidate interaction points to consider when attempting to explain how EO/S protocols may influence reproductive performance events. It will also be critical to include unique species-specific nuances and not draw conclusions from a generalized biological model.

The uterine cervix is the most caudal portion of the bicornuate uterus of ruminants and the first physical challenge to TrAI.[1] The goat cervix requires somewhat more technical ability than necessary to inseminate cows although it represents less of an obstacle than the cervix found in sheep.[8-10] In the non-pregnant healthy caprine, the cervix contains approximately four to six fibrous overlapping internal tissue flaps (annular folds) [3] commonly referred to as "rings" as portrayed in photo image 1 of a dissected cervix originated from a healthy 4 y old Alpine goat.

Similar to the ovine cervix[10, 11] the cervix of goats varies in length with breed, age, season and stage of the estrous cycle.[12] On average the cervix measures 5.73 ±0.35 cm in length and 1.60 ±0.19 cm in breadth.[13] A mean length of 2.59 ±0.61 cm and 1.07 ±0.17 cm in diameter was recorded for adult, non-gravid Red Sekoto goats of Nigeria.[14] In

comparative anatomical studies[15, 16] it has been reported that caprine cervices were less tortuous than their counterpart ovine cervices and that the goat had annular folds presenting a more concentric alignment. Prepubertal goats (4 and 8 m old) induced to estrus revealed a significant increase in the length and width of the cervix. [3]

Previous unpublished AI results generated in-house leads to the hypothesis that the site of semen deposition is influenced by the type of E/OS protocol used and that short P4 exposure influences the degree of cervix relaxation. Hence, the objective of this study was to evaluate if the site of semen placement depends on the hormone E/OS protocol selected and to determine if the cervix response in cycling goats is influenced by P4 exposure time when combined with different dosage of concurrent use of eCG and hCG as components of E/OS protocols for fixed-time TrAI breeding.

Materials and Methods

Animals

This study was conducted under field and research facility conditions using the guidelines of the Animal Care and Use Committee at the American Institute for Goat Research (AIGR), Langston Oklahoma (*Lat*. 35.945° N *Long.* -97.255° W, 292 m.a.s.l.) during the breeding seasons (September through January) of 2007 and 2008 along with their respective kidding seasons.

The study incorporated unselected mature and young goats of three breeds: Alpine (A); n= 50, Boer (B); n= 35, Tennessee Stiff Legs (SL); n= 21 and various percentage Boer × Spanish phenotypic crosses (*i.e.,* ½, ¾, and $^{5}/_{8}$) hereafter referred to as cross-breds (XB); n= 107. Goat ages ranged from1.5 to 10 y of age. The overall average age and standard deviation at breeding time was calculated to be 3.7 ±1.4 y. Likewise, the average and ±SD of body weights varied for all 4 age groups but as a whole it was 49.1 ±9.6 kg and the most common body condition score (BCS) on a scale of 1 to 5 was 2.5.

Animal management

The Alpine herd consisted of non-lactating goats managed semi-extensively on Bermuda or Sudan grass as well as being placed in wheat pastures when fresh forage was available. Nutritional supplementation was given when necessary using a dry-goat ration (ME 2.3 Mcal/kg and TP 14.5%).

The meat and fiber goat herd composition was also that of dry animals during the breeding season with exposure to the natural decreasing photoperiod characteristic of the fall season. All goats were managed extensively on native Oklahoma mixed grasses and

wheat pasture when fresh forage was available. As needed, goats were supplemented with either a low or high protein commercial custom-manufactured goat pellet supplement (Stillwater Milling Co. Stillwater OK) containing 13.3 or 20.3% CP, respectively. Hormone dosing and artificially inseminating was done in indoor facilities. All goats were provided fresh water and had free access to mineral supplement licking blocks. All goats were under veterinary care and were treated regularly for internal parasites with anthelmintics (*i.e.,* Cydectin® Fort Dodge, Animal Health. Fort Dodge, IW or Valbazen® Pfizer, Animal Health. Exton PA or Levazole® Schering-Plough Animal health Co. Summit, NJ), all had access to portable plastic or metal shelters. Goats were cared for and monitored daily by farm personnel.

Estrus/ovulation synchronization (E/OS) protocol

Goats were randomly assigned to an E/OS treatment group or to a control non-synchronized cohort. As depicted in Figure 1, goats allocated to different E/OS protocol groups received an intravaginally placed silicone elastomer CIDR-G® containing 300 mg of P4 (Eazi-Breed CIDR, Pharmacia & Upjohn Ltd. Rydalmere, New Zealand) allowed to remain *in situ* for different amounts of time.

Hormonal protocol for E/OS also included an i.m. dose of PG600® (Intervet Inc. Millsboro, DE) at 5 mL (400 IU of eCG and 200 IU of hCG) or 1.75 mL (140 IU of eCG and 70 IU of hCG) given 24 h prior to CIDR removal and 2 mL of Lutalyse® (Pfizer Inc. New, NY) containing dinoprost tromethamine equivalent to 5 mg dinoprost/mL given i.m. immediately after CIDR removal.

CIDR's were monitored daily to ensure retention. In the event that a devise was expunged it was replaced immediately. Comparison (control) groups did not receive any hormonal treatment.

Fixed-time breeding

All goats assigned to an experimental E/OS protocol and breeding procedure group were bred 48 to 50 h after removal of P4 using TrAI standard procedures (see detail below) or by fixed-time (48 to 50 h) natural service of penned goats. Breeding was attempted whether or not overt estrus signs were manifest. All bred goats were placed 5 to 7 d before their next scheduled estrus with bucks fitted with a breeding marking harness.

Goats assigned to the TrAI-pass treatment adhered to the same inseminating protocol as the TrAI group with the difference that no semen was used. These 'dry inseminations' were implemented to increase sample size to better evaluate the amount of time necessary to get through the cervix.

Goats on the non-estrus synchronized (NSync) control group were assigned to natural service and were bred on a NSync, spontaneous occurring estrus 24 h after the onset of standing estrus as determined from raddled marks. Female goats were removed from the breeding group as soon as a breeding mark was observed.

Transcervical artificial insemination (TrAI)

TrAI and TrAI-pass were performed by two experienced technicians. Of the 213 goats used in this study, all were inseminated using standard TrAI procedures[61] except that in 126 of the procedures (TrAI-pass) no semen was used.

The group of goats that got bred (i.e., using semen) by TrAI was inseminated using commercially purchased thawed-frozen semen which originated from 37 sires (Boer and Alpine breeds) and one lot of custom frozen semen collected in previous years from one Tennessee Stiff-Leg breed stud sire. Insemination was achieved using straws containing 0.5 mL of semen at a concentration of 1×10^6 and 1.2×10^6 sperm/straw (Reproductive Enterprises, Stillwater, OK or Bio-Genics Ltd. Salmon, ID, respectively). Non-TrAI or partially TrAI (i.e., no successful passing thru the cervix) were identified by the number of cervical annular folds (rings) that the AI instrument tip was able to overcome in its path to the uterine body, as judged by the AI technician. TrAI was performed using only one semen straw per inseminated goat which was randomly selected (within breed) from the freezer nitrogen tank.

Study variables

Independent variables

Primary independent variables (treatments) considered were: 1) P4 exposure **Extra-long** (XL): 24 d; **long** (L): 10, 12, 13 or 14 d; **short** (S): 5 d and **none** (N). 2) Dose level of chorionic gonadotropin (eCG/hCG): None (control), 140/70 units or 400/200 units, respectively. For clarity variables in this study have been organized in Figure 2.

Covariates. Secondary independent variables (blocking covariates) evaluated were: Goat breed as described previously: A, B, SL, and XB, TrAI technician (2), female age as a continuous or as a categorical ordinal variable (**1:** ≤3 years; **2:** >3 and ≤4; **3:** >4 and ≤5; **4:**>5, parity (nulliparous, primiparous and multiparous), type of fixed-time breeding:

Trans-cervical artificial insemination (TrAI) or trans-cervical passage with no semen delivery (TrAI-pass), and year of breeding/kidding; 2007/8 and 2008/9 respectively.

Dependent variables

The dependent (response) compound variable studied was uterine cervix tautness/relaxation which was evaluated through: time to traverse the cervix, site of semen placement, and TrAI success rate.

Time to traverse the cervix. Cervical relaxation was evaluated by the time (m) needed to traverse the cervix with the AI gun through the traversal cervical lumen following the application of a standardized procedure for timing cervical penetration as follows: The time elapsed at each TrAI (with semen) or TrAI-pass (with no semen) was recorded independently by different personnel than the inseminating technician. The chronometer was started when the lighted instrument entered the pre-inserted intravaginally-placed speculum and timing was stopped after traversing the cervix. A maximum of 5 m was allotted to try to pass through the cervix. When the 5 m mark was reached, semen was delivered (or shammed delivered in the case of TrAI-pass) in the area the inseminating gun was located in the reproductive tract at that moment.

Site of semen placement. Site of semen deposition refers to the vaginal or uterine region where semen was placed while attempting to cross the cervix up to a limit in time of 5 m. If the cervix was completely spanned the semen was said to be have been deposited trans-cervical (*i.e.,* in the uterine body). An unsuccessful inseminating attempt was defined as: an insemination that was not fully transcervical. In such case semen was deposited in the

vaginal vestibule close to the external os cervix or, if the cervix was threaded, in the cervical region accessed as determined by the number of folds crossed.

TrAI success rate

TrAI success rate (SR) was determined by: a) TrAI mechanical/ technical SR ($TrAI_{m/t}SR$), and b) TrAI biological SR ($TrAI_bSR$).

a. TrAI mechanical/technical success rate ($TrAI_{m/t}SR$)

The mechanical/ technical percent success rate (SR) for TrAI -within a given breeding protocol, was determined by the quotient between the number of successful timed TrAI and the total number of TrAI attempts:

$$\boldsymbol{TrAI_{m/t}SR} = \left(\frac{\text{N° of TrAI}}{\text{Total N° of insemination attempts}}\right) \times 100$$

where,

TrAI represents only inseminations able to fully cross the cervix (*i.e.,* transcervi-cal regardless of the number of rings crossed in the process). The total includes all insemination attempts that completely crossed the cervix and those that did not cross the cervix (*i.e.,* ∅Rings, 1R, 2R…nR) as determined and reported by the inseminating technician at time of insemination.

b. TrAI biological success rate ($TrAI_bSR$)

The biological percent success rate (SR) for TrAI was evaluated in terms of the conception rate (CR) and kidding rate (KR) obtained in the course of this study.[a]

To that end, the depth of insemination (number of cervical rings crossed) was associated with conception or with kidding per site of semen deposition.

$$\boldsymbol{TrAI_bSR} = \left(\frac{\text{N° of TrAI} \times \text{CR}_{\text{TrAI}}}{(\text{ØR} \times \text{CR}_{\text{ØR}}) + (1\text{R} \times \text{CR}_{1\text{R}}) + (2,3,4\text{R} \times \text{CR}_{2,3,4\text{R}}) + (\text{TrAI} \times \text{CR}_{\text{TrAI}})}\right) \times 100$$

where,

TrAI is a transcervical insemination, CR represents the conception rate (or, in turn, kidding rate [KR]; not shown in formula) resulting from a given type of insemination of the same characteristics, and ØR through 4R is associated with the specific number of rings traversed at AI. For example: 2, 3, 4R represents the total number of inseminations where the inseminating gun was able to get to the 2nd or 3erd or 4th cervical ring. Likewise $CR_{2,3,4R}$ represents the CR attained for all inseminations where the inseminating gun was able to deposit semen in the 2nd or 3erd or 4th cervical ring.

Depth of cervix surpass was evaluated by the inseminating technician by counting the number of rings crossed while performing TrAI and TrAI-pass.

[a] Conception rate was calculated as the number of goats conceiving (pregnant + aborted + embryo death) divided by total number of goats bred. Kidding rate was determined at parturition by dividing the total number of kidding females by the total number of females bred.

Statistical Analysis

Sample size

Treatment effects were analyzed using data generated by 186 goats that were bred once and 27 goats that were bred twice. Hence, of the 213 breeding goats, 186 (87**%)** observations were unique and 13% of the goats were evaluated more than once (*i.e.,* goat was rebred by NSyncp if open, or the same goat was used in a subsequent estrus).

Statistically analyzed data

Central tendencies were expressed as the arithmetic mean ± standard deviation (SD) or ± standard error (SE), as appropriate. All mean differences were considered statistically significant if the *p*-value was less than 0.05 unless otherwise stated.

Analysis of differences between proportions, in terms of comparative outcome of several 2×2 Tables for different classes of the covariate of interest, adjusted for the magnitude of the proportions of occurrences being compared, were performed by means of odds ratios (OR) as follows.[17, 18]

$$\boldsymbol{OR} = \frac{p_1/(1-p_1)}{p_2/(1-p_2)} = \frac{p_1/q_1}{p_2/q_2} - \frac{p_1 \times q_2}{p_2 \times q_1}$$

where,

an OR of 1 indicates that X and Y are independent and that the probability of an event can be expressed in terms of their marginal probabilities. That is, the condition or event under study is equally likely to occur in both groups. An OR greater than 1 indicates that the condition or event is more likely to occur in the first group. And an OR less than 1 represents that the condition or event is less likely to occur in the 1st group compared to

the probability of occurring in the 2nd group.[19] Where necessary, conversion from an OR to probabilities was performed as follows. [2, 20]

$$OR = \frac{\text{Probability}}{1 - \text{Probability}} \qquad and, \qquad Probability = \frac{\text{OR}}{1 + \text{OR}}$$

Statistical inference for odds ratios OR significance between P4 treatments, within breeding procedure, were analyzed by use of the Chi-square (χ^2) test with P values corresponding to two sided tests; with one degree of freedom, (JMP, 2011).[21]

$$\chi^2 = \frac{((|\text{Observed Frequency} - \text{Expected frequency}|) - 0.5)^2}{\text{Expected frequency}}$$

Statistical model

Ordered categorical data was fitted to an ordinal logistic polytomous ordinal model:[22, 23]

$$\hat{P} = \frac{e^{b_o + b_1 x_1 + b_2 x_2}}{1 + e^{b_o + b_1 x_1 + b_2 x_2}}$$

where,

$\hat{P}$ is the probability for the presence of the characteristic of interest (i.e., pregnant)

e represents the base of the natural logarithm.

b_o is a regression coefficient representing the Y axis intercept of the regression equation.

b_1 represents one of the regression coefficients defining the slope of the relationship.

X_1 represents one of the set of predictors.

The computerized analysis uses the maximum-likelihood method to fit a regression line to logit transformed data and the assumption of a proportional odds model was verified (UCLA Academic Technology Services, 2010).[24] The model's goodness of fit was quantified by comparing the observed counts to the estimated expected frequencies using a Chi-square (χ^2) statistic of the Pearson form.[25] Evaluation for the proportional odds assumption for the ordinal logistic model was done using an R language application (UCLA, 2010).[24]

The test of bioequivalence used was based on the Hauck-Anderson parametric method[26] implemented in the EquivTest™ software (Statistical Solutions Ltd. 2001). [27]

Time to traverse the cervix

Time to traverse the cervix was the event of interest and was evaluated using the semi parametric regression Cox proportional hazard model where the survivor S(o) and hazard function H(t) estimates are obtained, respectively, from: [28]

$$\boldsymbol{S_o} = \mathrm{e}^{(-\mathrm{H(t)})}$$

and,

$$\boldsymbol{H_i(t)} = \sum_{\mathrm{j}:\mathrm{t_j}<t} \frac{\mathrm{d_j}}{\sum_{\mathrm{I}\varepsilon\mathrm{R}} \mathrm{e}^{\mathrm{x_j}\beta}}$$

The hazard function H(t), determines the probability of having spanned the cervix a certain time after the start of the i^{th} inseminating attempt given that complete TrAI had not yet occurred. The baseline hazard function, $H_O(t)$, was the probability of crossing the cervix at time t, when all the independent variables in the model were at their base line

(mean) values.[29] The β_j are the estimated regression coefficients for the X_j independent variables (*i.e.,* P4 exposure, PG600, age group, site of semen deposition and parity) as previously determined to be important when fitting the data to a lsm model.

In the type of survival model used the time to traverse the cervix was considered the failure time random variable which had a staggered entry and right administrative censoring. The time scale used is in minutes (m) from the time origin given by the start of Tr-AI or TrAI-pass. Statistical significance was presumed when $P \leq 0.05$ (two-sided). Analyses were performed with JMP V.9 (SAS, 2011).[21]

Individual effects of each independent variable were evaluated with risk ratios (RR).[29] In this study a RR describes the relative risk of a goat not having a successful TrAI based on the comparison with event rates influenced by relevant independent variables. The risk ratio range (RRR) shows the change over the whole range of the regressor and is calculated as follows: [21]

$$\boldsymbol{RRR} = e^{PE(X_{max} - X_{min})}$$

where,

e represents the natural base number.

PE is the parameter's estimate of a given independent variable.

X_{max} is the maximum time recorded for a given treatment comparison.

X_{min} is the minimum time recorded for a given treatment comparison.

Data adjustments

Because some categories were empty or had few observations, invalidating statistical analysis assumptions, values depicting the crossing of cervical folds (rings) 2R, 3R and 4R were combined into one category (234R) before applying the ordinal logistic model to the TrAI and TrAI-pass data (total n= 213). Therefore, a total of 4 levels of cervical penetration were used *i.e.,* ∅, 1, 234, and TrAI. This adjustment resulted in a frequency of responses of: ∅R= 48, 1R= 16, 234R= 15 and TrAI= 134.

Results

Goats of different breeds, parity and ages used in this study had an overall average weight at first breeding of 49.1 ±9.58 kg (Figure 3).

Cervix response to the E/OS protocols was evaluated for potential influence on the technical/mechanical and biological success rate attained when performing TrAI.

TrAI mechanical/technical success rate ($SR_{m/t}$)

In this study successful inseminations are defined as those inseminating attempts where the AI technician was able to fully penetrate the uterine cervix irrespective of the number of rings crossed. The calculation of success rates included both TrAI and TrAI-pass groups (n=213) since there were no differences that could be attributed to the breeding procedure (P>0.07) (Table 1). Likewise, results were not influenced by AI technician where each technician had equal percentage of successful inseminations (63%; P>0.995). The corresponding observed confidence ± 5% bounds (-0.1154, 0.1146) were within equivalence limits for the difference between inseminators (P<0.002) but not for the upper bound of the observed confidence ± 5% bounds (0.0071, 0.2365) equivalence limit for the difference between type of breeding (P>0.1369).

Synchronization protocol had an influence on TrAI $SR_{m/t}$ which is dependent on the length of time goats were in contact with P4 (see Figure 4). The 12 d P4 exposure protocol with a TrAI $SR_{m/t}$ of 75% was greater (P<0.02) than when goats were inseminated during a natural estrus with no hormone synchronization protocol with a TrAI $SR_{m/t}$ of 68%. The 12 d P4 protocol performed better yet when compared to the other two P4 exposure protocol extremes, it had 2.8 and 3.2× greater likelihood of a

successful TrAI than the short 6 d P4 exposure protocol with 52% TrAI $SR_{m/t}$ ($P<0.005$) or the 48% TrAI $SR_{m/t}$ ($P<0.016$) obtained with the 24 d XL P4 exposure protocol.

Other main effect comparisons showed that there were differences between the non-treated control goats when they were compared with the short 6 d P4 protocol at 68% $SR_{m/t}$ (2.0× OR; $P<0.0001$) and when compared with the 24 d XL P4 exposure protocol at 48% $SR_{m/t}$ (2.3× OR; $P= 0.014$).

Both extreme P4 exposure protocols, that is the 6d S protocol and the 24 d XL protocol, did not differ between each other ($P= 0.760$). However, the observed confidence bounds for equivalency (-0.2541, 0.1819) were not within equivalence limits for the lower 5% bound ($P>0.1160$). Hence, despite the absence of a statistically significant difference between both protocols, there is also insufficient evidence to claim that the P4 exposure protocols were similar.

TrAI biological success rate (SR_b)

The biological success rate evaluation was used only on goats TrAI'ed with semen (n=87). And, as explained in the Material and Methods section, SR_b incorporated in its assessment both conception and kidding rates obtained throughout this study as tabulated in Table 2.

Results of the OR calculations for both mechanical/technical and biological success rates are given on Table 3. Despite the significant effect between differences in synchronization protocols observed for the $SR_{m/t}$, none of the protocols influenced the biological success (*i.e.,* CR or KR) in terms of the ability to successfully traverse the cervix and result in different conception and birth rates. As explained in Material and

Methods section, SR_b could not be tested for equivalence because success and failure results were adjusted by actual pregnancy or kidding rates. These mathematical rate adjustments generated decimal number results which could not be evaluated with the equivalence test software used.

Cervical tautness/relaxation

Cervical tautness/relaxation was evaluated by depth of cervix penetration at fixed-timed insemination (*i.e.,* counting the number of cervical folds crossed while performing TrAI or TrAI-pass) and by assessing the time needed to traverse the cervix.

Site of semen placement

Depth of cervix surpass is synonymously used as "site of semen placement". As explained in Materials and Methods, since some number of rings crossed categories were empty or had sparse observations; invalidating statistical analysis assumptions, values depicting the crossing of cervical rings 2R, 3R and 4R were combined into one category (234R) before fitting the ordinal logistic and the Cox proportional hazards model to the TrAI and TrAI-pass data. Therefore, a total of 4 levels of cervical penetration categories were used *i.e.,* ∅, 1, 234, and TrAI. This adjustment resulted in a frequency of responses of: 48 ∅R, 16 1R, 15 234R and 134 TrAI.

The starting saturated logistic regression model used was:

$$[\mathbf{1}] \qquad \boldsymbol{Site} = \mathrm{P}_4\, exposure + \text{Breeding procedure} + \text{PG600} + \text{Breed} + \text{Parity} + \text{AI Tech.} + \text{Age cohort} + \text{Breeding year}$$

Using this model (omnibus test of P<0.0005 with LOF>0.999) it was concluded that neither breeding procedure (P>0.0727), PG600 (P>0.3555), breed (P>0.1287), AI technician (P>0.7766), age cohort (P>0.7001), or breeding year (P> 0.0744), had an influence on the logistic model designed to predict site of semen deposition. For this reason, the model was abridged to one with simpler structure by eliminating factors that did not contribute significantly to explain the overall observed variability or that were increasing the LOF of the data to the postulated model. The most efficient reduced model (P<0.0007) with the least lack of fit (χ^2= 77.38; P>0.8623) was given by:

[2] ***Site*=** P4 Protocol + PG600 + Parity + Age Cohort

Age cohort, just as in the full model, did not show a significant contribution (P>0.5337), its inclusion as part of the streamlined model improved the model's effectiveness by removing some of the variability allowing PG600 to become significant and age cohort to become more relevant. That is, a change in significance from (P>0.3555) to (P<0.0597) and (P>0.7001) to (P>0.5337), respectively. The effect of each independent variable, included in the reduced model [2], on the predicted profiles of semen site deposition is given in Figure 4 and the parameter estimates associated with said model are given in Table 4.

Predicted profiles of treatment effects

Each panel in the profile diagram of Figure 4 displays an independent (x) variable profile trace of a complex model surface for the effects of a given treatment. A profile trace represents the predicted response as one variable is changed and the remaining variables in the model remain constant at their stated initial value. The set of profiles given in

Figure 4 are the result of one such set of conditions, out of 144 possible combinations (data not shown), chosen to exemplify results of interest.

The particular panel of profiles chosen depicts the characteristics of a control non-synchronized goat. In this particular case it can be seen that 61% of the inseminations would be inside the uterus (successful or TrAI) and 22% in the vaginal vestibule when a maiden young goat (≤ 3 y of age) receives no synchronization hormones. The model shows that the number of TrAI's would increase with increasing P4 exposure ($P<0.03$). PG600, on the other hand has a tendency to reduce the number of successful inseminations ($P<0.06$). As expected the greater the parity the more likely a goat is to have a TrAI ($P<0.01$). Whereas, the age category group where a goat belongs to had no influence on the site in the reproductive tract semen was deposited ($P>0.53$).

Time to traverse the cervix

On the basis of the perfunctory lsm procedure, time to pass through the cervix data was adequately described by the standard multiple regression model ($P<0.0001$) with no LOF ($P>0.3064$). However, various attempts to normalize the data by mathematical transformations (data not shown) to validate ANOVA proved unsuccessful. Hence, after verifying that time-to-event data was not normally distributed due to the presence of right censored data at 5 m, time to traverse the cervix was analyzed with survival analysis statistical techniques.[30, 31]

The clinical survival end-point was determined to be a successful TrAI. A plot of all the 213 time measurements is given in Figure 6, of these 130 (61%) were uncensored values and 83(39%) were administratively censored at 5 m. The y-axis shows individual

breeding attempts. A solid line not followed by a dashed line marked by an "x" at the end of the line indicates a successful event. A dashed line connecting to the right of a solid line with a left triangle indicates a right-censored event.

A univariate approach using Kaplan-Meir survival procedures yields information regarding descriptive statistics (*i.e.,* mean ±SE, median, and $CI_{95\%}$) for each of the independent variables isolated from other main effects as shown in Tables 5 through 9. These results can then be compared with the Cox proportional hazard regression modeling which includes confounding factors and other covariates as part of the model.

Multivariate data modeling

The starting saturated proportional hazards regression model used included the same independent variables used to analyze the response variable "depth of cervix surpass". Variables with the highest χ^2 probabilities were sequentially excluded from the model. That is, breeding year (P>0.9513), breed (P>0.8083), breeding procedure (P>0.3517), and AI technician (P>0.3088). PG600 which had a (P>0.9107) was retained as a possible source of variation modifier.

The reduced model yielded a χ^2= 226 (P<0.0001) and was given by:

[3] ***Time*** = P4 Protocol + PG600 + Parity + Age Cohort +Site

Using reduced model [3] it was concluded that only PG600, just as in the full model, did not show a significant contribution (P>0.9459). The corresponding parameter estimates are given in Table 10.

Whereas in the Kaplan- Meir univariate analysis the goat grouping according to age turned out to be not significant, using the Cox proportional hazard's model and the influence of covariate terms it was demonstrated that in fact age is a factor that influences ($P<0.0268$) the time at which TrAI inseminations can be accomplished when the rest of the covariates remain constant at their mean baseline values.

Additionally, when PG600 was compared using both univariate and multivariate-based models it was determined that as an isolated variable it was influential (LR: $P<0.0265$ and W: $P<0.0476$) but that when considered among the covariates of the reduced model its influence was made irrelevant ($P>0.9459$).

The basal survival model using the Cox proportional hazard model applied to the data modified by all five covariates (P4 exposure protocol, PG600 dosage level, age grouping, goat parity and cervical site of semen placement) is given next in Figure 7.

Chi-square (χ^2) values and probabilities for all sources of variation are the same in both [a] and [b] except for the variable Site adj. where χ^2 changes from 189.6 to 24.6. However, in both model [a] and [b] the probability for the effect of site of semen placement remains as ($P<0.0001$).

Individual covariate effects

The significant individual effect of each independent variable is presented next.

E/OS protocols based on different P4 exposure time

A Failure Plot (proportion of goats TrAI'ed) reverses the y-axis to show the number of failures rather than the number of survivors (as given in Figure 7 [a] and [b]) as has been the tradition in the Reliability literature which depicts survival plots rather than failure

plots. The length of time goats were exposed to P4 had an influence (P<0.0108) on the time it took to penetrate a goat's cervix (see Figure 8). As a group all goats had a median TrAI time of 3.08 m.

Figure 8 also depicts the median value where 50% of goats that were not treated (N) or that were estrus/ovulation synchronized using P4 for 12 to 14 d were TrAI'ed in about 2.4 m, whereas it took almost twice longer to TrAI'ed 50% of the goats that received P4 for 5-6 d or for 24 d.

As depicted in Figure 9, when the TrAI response was compared among the P4 exposure treatments, the short P4 exposure protocol had a risk ratio (RR) always smaller than all other treatments including goats that were not synchronized (P<0.05). The short P4 exposure protocol had a 3.0 RR compared to the 24 d protocol, a 2.6 RR when compared to goats that were not synchronized and a 1.8 RR when compared to the 12-14 d protocol.

Although the x-long P4 exposure protocol had numerically greater RR's than all the rest of the treatments, goats that received the long or the extra-long P4 exposure protocol were not more likely to be TrAI than animals that were not E/OS (P>0.05).

Goat age grouping

The age group to which goats were assigned in this study influenced (P<0.0268) the time it took to penetrate a goat's cervix (see Figure 10). As a group all goats had a median TrAI time of 3.03 m.

Figure 10 depicts the median value where 50% of goats that were categorized in the four age groups were successfully inseminated. Older goats (5 y) were more likely to get

inseminated earlier at about 2.5 m after the procedure was initiated rather than 3.9 m and 2.8 m for younger goats at >4 and ≤5 y and >3 and ≤4 y, respectively.

Fifty percent of the young goats (≤3 y) were inseminated about 1.8 m later (a change of 42%) than it took to TrAI goats 5 y or older.

As evidenced in Figure 11, the widest margin in the RR difference found in attaining the animals inseminated was found between the oldest goats and those categorized amid 4 and 5 y old. Older goats, compared to other age groups, were always more likely to become inseminated; however, the RR became smaller with increasing age difference. The direction of RR of TrAI was always from older groups to younger groups except in one circumstance where the younger goats presented 2.6× the probability of having a TrAI quicker than goats in the 4 to 5 y age range.

Goat parity

The grouping to which goats were categorized in terms of parity influenced ($P<0.0028$) the time it took to completely penetrate a goat's cervix (see Figure 12). As a group all goats had a median TrAI time of 3.03 m.

Half of the primiparous goats were likely to have had transcervical insemination almost 3 m sooner and almost 2 m before than 50% of nulliparous goats ($P<0.003$). Primiparous and multiparous goats were not different in respect to the median number of goats inseminated at a given time ($P>0.05$).

As shown in Table 11, the time to penetrate the cervix was likely to be 2.5× shorter for primiparous goats as compared to goats that had never kidded. Primiparous goats were also 1.65× more likely to have a transcervical insemination than goats that had kidded

more than once. Multiparous goats were also 1.5× more likely to have a TrAI as goats that had never kidded.

Cervical site of semen deposition

The site where semen was placed in the goat's cervix influenced ($P<0.0001$) the time to completely penetrate a goat's cervix (see Figure 13).

The step function corresponding to ∅R and 1R is biased. For this reason both cumulative distributions do not appear in the failure plot as both had all their observation at 0 value. By definition, besides the trans-cervical inseminated goats, none of the other goats were able to be TrAI'ed. Fifty percent of the goats that had a successful insemination were finished in 1.8 m ($P<0.0001$),

Risk ratios for the site where semen was deposited in the cervix have no straight-forward interpretation they are provided in Table 12 only for completeness of analysis.

PG600 dose level

As can be seen in Figure 14, both the control (no PG600) group and the group of goats receiving 5.0 cc of PG600 appear to be more efficient than the 1.75 cc PG600 dose protocol in that the latter almost doubled the time to TrAI. However, PG600 was not influential ($P>0.9459$). This result can also be verified by the fact that both germane confidence intervals (see Table 10): 95% $CI_{PG600\ [1.75-0]}$ (-0.826, 1.213) and 95% $CI_{PG600[5-1.75]}$= (-0.585, 0.421) include 0. This conclusion is more apparent when analyzing the RR for the PG600 dosage level comparisons in Table 12, where each comparison (ratio) is numerically very close to 1 (no difference).

Discussion

To attain maximum reproductive performance (RP), while maintaining genetic selective pressure and decreasing costs to get females bred, commercial goat producers use hormonal estrus/ovulation synchronization (E/OS) and fixed-time breeding. When goats are reproductively active E/OS can be accomplished by the exogenous provision of a luteolytic agent which will prematurely regress existing *corpora lutea*[32] or by using progestagens which are supplied to extend the luteal phase.[33, 34]

To design and evaluate hormonally E/OS procedures for more effective use of progesterone (P4) research has focused on ovarian follicle dynamics,[35, 36] rather than corpora lutea lifespan *per se*. Year-round breeding field trials have validated the use of P4 for 5 to 6 d in combination with a luteolytic dose at time of initiation of the P4 regime and equine chorionic gonadotropin (eCG) or estradiol benzoate (EB).[37-39] This short P4 exposure in lieu of the customary 12 to 14 d use since its inception[40] or 9 to 16 d[34] or even the longer 16 to18 d[41, 42] or 18 to 21 d[43] has attracted attention due to its time economy. In fact, the 5 to 6 d short-term protocol has been shown to induce similar P4 concentrations among treated goats and in combination with eCG or EB results in similar increase in estradiol-17ß and a comparable LH surge, inducing ovulation in 86.7% of treated females at a consistent ±60 h interval after the end of P4 exposure.[44]

Currently in the U.S. no reproductive hormone has been approved for goats since last reviewed.[45-47] This regulation limits the use of some pharmaceutically prescribed hormones to an "off-label" use as specified under the Food and Drug Administration Compliance Policy Guide.[48] The commercially available product PG600®, designed for porcine reproduction, is the only legally available pharmaceutical in the U.S. which

contains chorionic gonadotropins. PG600 is a mixture of 67% chorionic gonadotropin of equine origin (eCG) and 33% chorionic gonadotropin of human origin (hCG). PG600 has been sold in the USA since the 1990's for inducing estrus in prepuberal gilts and for abolishing anestrus in sows that do not return back to estrus after weaning.

When eCG is used in goats it characteristically elicits both luteinizing hormone (LH) and follicle stimulating hormone-like (FSH) activities.[49, 50] Because of this dual biological effect, eCG has been used to induce ovarian follicular growth, both for goat enhanced ovulation and for estrus induction and synchronized breeding programs.[51]

The clinical role of hCG has been comprehensively reviewed for dairy cows.[52] A similar analysis, in the context of goat reproduction, has not been found. Research on the use of hCG with goat/sheep has centered on the effects of hCG given at breeding time[53] or after breeding [54-58] and its role on embryo survival and reproductive hormone profiles.

The use of eCG in combination with hCG in lieu of eCG alone in goats has been scarcely addressed.[41, 59] Much of the information available regarding the concurrent use of eCG and hCG has been done in the species for which it has been developed (i.e., porcine) and to date a large body of studies are available which cannot be addressed in this review.[60-62] However, there has also been information generated with other laboratory[63] and companion animals,[64] wildlife,[65] and with other farm animals. Probably most useful information because of the phylogenetic relationship, comes from studies performed with sheep[66-71] and cows.[72]

Better understanding of physiology and animal behavior keeps motivating attempts to improve goat breeding performance using ART's. The progress in reproductive efficiency, has many fronts one of which remains the optimum use of E/OS protocols addressing several practical and economical concerns.[73]

When using E/OS procedures the goat industry has considered "natural" all non-pharmacologic means,[74] but overwhelmingly the focus has been using hormonal procedures. [35,37,44,75] Recently standard procedures in E/OS used in goat reproductive management has shifted interest to the use of a shorter period of P4 exposure on grounds of time economy and due to alleged improvements in the synchrony of estrus and ovulation and its ability to provide better pregnancy rates for timed artificial insemination.[43] Additionally, because of regulatory limitations on available pharmacology, a combination of eCG and hCG (PG600) was chosen to replace the use of eCG alone which has been the choice gonadotropin in earlier trials.[46, 76, 77]

In non-published work performed by the author and co-workers, the use of short P4 priming (5 to 6 d) in combination with the concurrent use of eCG/hCG and fixed time AI, successful behavioral estrus synchrony was accomplished but it appeared that TrAI was more difficult to achieve. This anecdotal evidence lead to interest in studying the use of different time of P4 exposure along with the concurrent use of eCG/hCG and fixed-time breeding and how these efforts may influence uterine cervix character.

Along with the perceived mechanical and/or technical hindrance mentioned, there was also the possibility that confounding effects were introduced by the use of different chorionic gonadotropins and fixed-time breeding. Hence, the objective of this study was

to develop an experimental design to ascertain the influence of E/OS methods where P4 was used for different lengths of exposure time and PG600 was also used at different dosage levels in lieu of only using eCG. A standard fixed-time across all treatments would minimize its potential confounding effect. Goat breed, season, AI technician, breeding procedure, parity, age and cervical site of semen placement were chosen as covariates that were included in the statistical models to take into account other potential sources of extraneous variation.

The statistical analysis of the data generated; i.e., time used to traverse the cervix under the different putative influential factors, required survival analytical techniques[30] because timed data usually does not follow a normal (Gaussian) distribution.[31, 78]

Although in this study no anatomical measurements of the cervix were recorded it is sensed that, by en large, the cervical structure was representative of healthy, non-pregnant goats with physical characteristics representative of an age range from 1.46 y to 9.86 y and mixed parity as portrayed in Figure 14 in panels [a] and [b] respectively. Nulliparous goats were maiden doelings believed to be all post-pubertal exposed to breeding in the 2nd reproductive season of their lives.

The number of cervical rings that an AI technician is able to traverse is a consequence of a goat's innate anatomical makeup, a combination of uterine physiological changes occurring during estrus, an indication of the mechanical/technical expertise of the inseminator and the time allotted to attempt intra-uterus semen deposition. Since goats bred were presumed to be in estrus, the evaluation of cervical response largely characterizes changes due to the relaxation of cervical structures, presumably under the

influence of estrogen and the preliminary synchronization protocol which elicited changes making cervical mucus to become more profuse and liquefied increasing lubrication of the cervical lumen as shown in other studies[79] and, consequently, ease of passage of the semen delivery inseminating instrument. Although there are no studies in goats regarding the cervical changes at estrus, it is expected from studies in other species that typically the cervix, under biochemical and neural control, will go through anatomical, histological and physiologic changes with some contraction, softening, dilating (opening) and perhaps even some degree of effacing (thinning) several hours before standing estrus, LH surge, ovulation, and fertilization.

The physical response of the cervix was studied indirectly in terms of events believed to be influenced by cervical relaxation. Where, cervical relaxation *per se* was determined by depth of cervical penetration at insemination and by the time taken to penetrate the cervix or overcoming a portion of the cervix up to a limit given by 5 m in time. A biological assessment was attempted *a posteriori* by means of associating semen placement in the cervix to actual conception rate and kidding results obtained in this experiment.

For the purposes of this study it was important to demonstrate that the two types of breeding procedures (TrAI and TrAI-pass) were independent and that, therefore, TrAI-pass observations could be used to augment the sample size of the germane observations gathered. In fact, the test of equivalence for breeding procedures performed supports this claim further. Likewise, the absence of significant differences between individual inseminators was anticipated as both technicians used standardized inseminating techniques. However, on this particular comparison of the influence by AI technician the equivalence test only supported the 5% CI lower bound.

Despite the significant effect between the differences in synchronization protocols observed for the $SR_{m/t}$, described above, none of the protocols influenced the biological success (*i.e.,* conception rate and/or kidding rate). These results imply that even if the ease or difficulty of insemination is increased by adopting a certain E/OS protocol, reproductive performance, evaluated by means of CR and/or KR, will not be influenced.

P4 exposure synchronization protocol

It is apparent from the hormonal synchronization protocol profile (see Figure 4) that the use of P4, for any length of time (*i.e.,* 6, 12 or 24 d), influences favorably the potential site of semen deposition in terms of whether or not semen could be placed trans-cervical compared to nulliparous goats (≤ 3 y of age) not hormonally treated (*i.e.,* P4 and/or PG600) that have a predicted successful TrAI breeding of 61.3%. Long P4 exposure protocol has the greatest effect with a predicted 98.3% of the inseminations being trans-cervical ($P<0.01$) when all other variables are held constant at the classifying category at which they yielded the greatest TrAI insemination (*i.e.,* PG600= 0, Parity= multiparous, Age Cohort≤ 3 y). Maintaining the above treatment conditions, a TrAI failure rate (percent of goats where no rings are crossed) of 22% would be anticipated.

The results also indicate that both short and extra-long P4 protocols will not have an effect on depth of cervical penetration ($P>0.98$) and ($P>0.26$), respectively.

PG600 dosage level

Besides the control group which did not receive any gonadotropins, two PG600 doses were chosen. A dose of 5cc (400 IU eCG and 200 IU hCG) was established to replicate what other experiments used in the previously.[10] The dose of 1.75 cc (140 IU of eCG and

70 IU of hCG) was selected to use a similar amount as has been used with eCG (i.e., 200 IU) in experiments that featured short (5-6 d) P4 protocols.

A negative effect on semen site placement was documented when PG600 was given at 1.75 cc compared to control animals receiving no PG600 ($P<0.06$). PG600 did not influence ($P>0.26$) cervical semen placement between goats receiving 1.75cc and 5cc.

Parity category

Goat parity grouping influenced ($P<0.0028$) the time taken to penetrate a goat's cervix. Nulliparous animals are predicted to have significantly less TrAI inseminations than primiparous or multiparous goats. No difference was found ($P>0.666$) between primiparous and multiparous goats in predicted site of semen deposition.

Age cohort

Although a consistent tendency is observed chronologically, that is there is less predicted TrAI accomplished with increasing age, statistically no difference was found with a probability range of ($P>0.20$) to ($P>0.98$) for the different age cohorts.

The length of time P4 was used to synchronize estrus influenced how long it took to penetrate a goat's cervix. It is well known of the interaction of P4 and the cervix.[80] The results obtained in this study may imply that the presence of exogenous P4 exposure increases the accessibility or promotes the expression of estrogen receptors that will interact with the heightened estrogen production at estrus or there could also be concomitant increases in receptors for the hormone relaxin as shown in the porcine cervix [81] or prostaglandin E and FSH on cervical penetrability in ewes.[82] Additionally it is known that estrogen dilates cervical tissue.[83]

The age group to which goats were assigned in this study influenced the time it took to penetrate a goat's cervix. This result has already accounted for the influence of parity as an independent effect so documented differences are explained solely by age anatomical development and reproductive events associated with age such as the number of estrous cycles each group has been through and other confounding factors such as litter size.

The results of site of semen deposition and the time to accomplish were biased and scarce in different portions of the cervix to draw any valid conclusions. However, previous studies had shown that pregnancy rates were a direct result of the depth at which semen is placed.[84-88] Our inseminating technique efforts to accomplish intrauterine semen deposition were motivated and influenced by the results of the aforementioned studies.

Nevertheless, in light of other recent research[89] which indicates a great deal of reproductive success with exo-cervical semen placement. The time allotted to inseminate in said previous work was one third to one fourth the average time used in this study. Because previous research has shown overwhelmingly that pregnancy rates increase with depth of insemination we conclude that further research is needed to address this particular apparent contradiction. Prolonged transcervical insemination efforts may in fact be triggering detrimental neuro-hormonal and stress-related behavioral events capable of meddling with oocyte and sperm transport as insemination and reproductive events are happening at the same time and perhaps detrimental carry-over effects capable of interfering with appropriate embryo developments to unfold at a later time.

Conclusion

This study focused on the effects that P4 exposure time and PG600 dose levels may have on uterine cervix response as characterized by a) Cervical tautness/relaxation (i.e., time to traverse the cervix and cervical site of semen deposition, and b) TrAI success rate (i.e., technical/mechanical and biological).

In this study depth of cervix surpass, which was greatest for non-synchronized goats, was influenced by: P4 time of exposure, and parity. Whereas the time necessary to penetrate a goat's cervix was influenced by: P4 exposure time, age, parity and cervical semen placement. There was not enough evidence to accept the hypothesized influence of PG600 on both the depth of cervix surpass and time necessary to traverse the cervix. Too long or too short P4 exposure influences negatively the physical character of the cervix. It took twice longer to TrAI 50% goats exposed to either extreme of P4. When the TrAI response was compared, the short P4 exposure protocol had a RR always smaller than all other treatments including NSync goats ($P<0.05$).

As expected, older than 5 y old goats were more likely to get inseminated faster. That is, 1.6× faster than younger goats and were always more likely to have a successful TrAI. Half of the primiparous goats were likely to be TrAI'ed 3 m to 2 m sooner than 50% of goats that had never kidded. The primiparous and multiparous goat cervices have the same physical vulnerability in response to the various E/OS protocols; they were not different ($P>0.05$) in respect to the median number of goats inseminated at a given time.

It stands to reason that the cervical site where semen was placed influenced the time it took to completely penetrate a goat's cervix. There is a direct relationship between site

of semen deposition and time to reach the site. That is the same as saying that the deeper the insemination is accomplished the longer it will take to achieve it.

The effect of P4 exposure protocol on the mechanical and/or technical success rate ($SR_{m/t}$) was favorable for the long 12-14 d protocol but influenced negatively both P4 protocol extremes. That is, both the 5-6 d P4 short protocol and the 24 d X-Long P4 protocol reduced significantly the ability to succeed in traversing the cervix. This result is important because recent published efforts in estrus synchronization protocols advocate reducing P4 exposure time on grounds of time economy and lack of negative effects on reproductive performance.

The observed decrease in breeding efficiency in some studies using fixed-time AI^{90} (also see Chapter IV of this document) may be explained by the increased difficulty of attaining full transcervical semen deposition because of negative effects of the E/OS protocol on the cervix anatomical character. The potential for indirect and/or direct effects on the biochemistry of the cervix or the triggering of a neuro-endocrine response, which ultimately hampers conceptus development, needs to be considered in new studies henceforth.

However, this study was also important in that it demonstrated that, at least with respect to conception and kidding rate, increased mechanical/technical impediment to traverse the cervix does not necessarily translate to deficient biological success rate. In light of other results shown in the preceeding chapters, this counterintuitive result needs further independent corroboration, as well as some non-biased measure to evaluate the validity of the mathematical formulas developed to characterise TrAI success rate.

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Table 1. Cervical ring penetration as a function of breeding procedure, P4 exposure and AI technician.

		Breeding Procedure								
		TrAI			TrAI-pass					
		P4 exposure protocol								
Tech.	**Site**	**L**	**N**	**S**	**L**	**N**	**S**	**XL**	**GTotal**	**%**
A	**0R**	2	1	--	4	1	7	7	22	22.4
	1R	1	1	--	2	--	2	2	8	6.9
	234R	--	1	1	1	--	2	1	6	7.8
	TrAI	12	2	9	17	6	9	6	61	**62.9**
A Total		15	5	10	24	7	20	16	97	45.5
B	**0R**	--	3	5	2	6	8	2	26	22.4
	1R	2	--	2	2	1	--	1	8	6.9
	234R	--	4	3	1	1	--	--	9	7.8
	TrAI	8	20	10	13	12	4	6	73	**62.9**
B Total		10	27	20	18	20	12	9	116	54.5
	GTotal	25	32	30	42	27	32	25	**213**	
		61/87=70%[a]			73/126=58%[b]					

L:12-14 d; **N:** None; **S:**5-6 d and **XL:** 24 d.

TrAI: Transcervical AI; **TrAI-pass:** TrAI with no semen delivered.

Site: 0R, 1R or 2,3,4 corresponds to number of cervical rings traversed.

[a-b] ($P<0.07$).

Table 2. Conception, pregnancy and kidding rates matching the reproductive tract site of semen placement.

	Number of cervical rings crossed				
	0R	**1R**	**2-3-4R**	**TrAI**	**Total**
Pregnant/Kidded	--	1/1	2/2	16/12	19/15
Aborted	--	--	--	1	1
Embryo death	3	--	3	9	15
Open	8	5	4	35	52
Total goats	11	6	9	61	87
Conception rate[a]	27.3%	16.7%	55.6%	42.6%	40.2%
Pregnancy rate[b]	--	16.7%	22.2%	26.2%	21.8%
Kidding rate[c]	--	16.7%	22.2%	19.7%	17.2%

[a]N° of goats conceiving (pregnant + aborted + embryo death)/ total goats bred.
[b]N° of goats pregnant (45 d dPB by ultrasound imaging)/total goats bred.
[c]N° determined at parturition: N° of kidding females/total goats bred.

Table 3. TrAI mechanical and biological success rate odds ratios (OR) by P4 protocol.

Mechanical/technical Success Rates			Biological Success Rates					
			Conception rate			*Kidding rate*		
			OR=1.4×			OR=1.3×		
			P=0.0140			**P=0.8316**		
L vs N	**75%**	**68%**	**L vs N**	**83%**	**72%**	**L vs N**	**86%**	**83%**
	50.0	40.0		21.0	16.8		9.9	7.9
	17.0	19.0		4.5	6.7		1.6	1.7
			2.8×			1.9×		
			P=0.0067			P=0.5697		
L vs S	**75%**	**52%**	**L vs S**	**83%**	**59%**	**L vs S**	**86%**	**76%**
	50.0	32.0		21.0	13.4		9.9	6.3
	17.0	30.0		4.5	9.4		1.6	2.0
			3.2×			1.9×		
			P=0.0154			P=0.6912		
L vs XL	**75%**	**48%**	**L vs XL**	**83%**	**60%**	**L vs XL**	**86%**	**77%**
	50.0	12.0		21.0	5.0		9.9	2.4
	17.0	13.0		4.5	3.5		1.6	0.7
			2.0×			1.5×		
			P=0.0000			P=0.7294		
N vs S	**68%**	**52%**	**N vs S**	**72%**	**59%**	**N vs S**	**83%**	**76%**
	40.0	32.0		16.8	5.0		7.9	6.3
	19.0	30.0		6.7	3.5		1.7	2.0
			2.3×			1.4×		
			P=0.0140			P=0.8159		
N vs XL	**68%**	**48%**	**N vs XL**	**72%**	**60%**	**N vs XL**	**83%**	**77%**
	40.0	12.0		16.8	5.0		7.9	2.4
	19.0	13.0		6.7	3.5		1.7	0.7
			1.2×			.0×		
			P=0.7604			P=0.9814		
S vs XL	**52%**	**48%**	**S vs XL**	**59%**	**60%**	**S vs XL**	**76%**	**77%**
	32.0	12.0		13.4	5.0		6.3	2.4
	30.0	13.0		9.4	3.5		2.0	0.7

×= Number of times that a given P4 exposure protocol will be more likely to yield a successful TrAI.

P= Represents the odds ratio probability of a chance occurrence.

Treatments: **N** control group with no hormonal synchronization (goats bred in natural estrus);
S short (6 d P4); **L** long (12 d P4) and **XL** extra-long (24 d P4).

Table 4. Parameter estimates for independent variables used in the ordinal logistic model describing the site of semen placement.

Term	Estimate	Std Error	χ^2	Prob > χ^2
Intercept [TrAI]	1.595	0.944	2.85	**0.091**
Intercept [1R]	1.980	0.948	4.36	**0.037***
Intercept [234R]	2.400	0.952	6.35	**0.012***
Protocol [None]	-1.135	0.915	1.54	0.215
Protocol [Short]	-0.049	0.373	0.02	0.895
Protocol [Long]	1.175	0.456	6.63	**0.010***
PG600 [1.75 - 0]	-2.215	1.100	2.41	**0.065**
Pg-600 [5 - 1.75]	0.574	0.505	1.29	0.255
Parity [Primiparous - Nulliparou	1.600	0.441	11.88	**0.001***
Parity [Multiparous - Primiparou	-0.261	0.604	0.19	0.666
Age cohort [B2 - A1]	-0.563	0.445	1.60	0.206
Age cohort [C3 - B2]	-0.388	0.666	0.34	0.560
Age cohort [D4 - C3]	0.018	0.662	0.00	0.978

*Statistically significant comparison.

Table 5. Effect of P4 exposure protocol.

Descriptive statistics using Kaplan-Meir survival procedures.

P4 Protocol	N° TrAI	Number censored	Mean	Standard Error	Median Time	Lower 95% CI	Upper 95% CI
L (12-14 d)	47	20	2.556	0.200	2.33	1.75	3.08
N (no synchr.)	41	18	2.537	0.189	2.42	1.98	2.67
S (5-6 d)	30	32	3.654	0.198	---	3.37	5
XL (24 d)	12	13	2.829	0.308	---	1.83	5
Combined	130	83	3.026	0.118	3.08	2.47	3.73

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	12.1357	3	0.0069*
Wilcoxon	12.7897	3	0.0051*

Table 6. Effect of goat age grouping.

Descriptive statistics using Kaplan-Meir survival procedures.

Age Group	N° TrAI	Number censored	Mean	Standard Error	Median Time	Lower 95% CI	Upper 95% CI
≤3 yr	33	26	3.419	0.209	4.33	2.83	5
>3 and ≤4	61	31	2.764	0.176	2.83	2	3.37
>4 and ≤5	17	15	2.797	0.256	3.89	1.83	5
>5 yr	19	11	2.145	0.206	2.5	1.27	5
Combined	130	83	3.026	0.118	3.08	2.47	3.73

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	3.7928	3	0.2847
Wilcoxon	4.9021	3	0.1791

Table 7. Effect of goat parity category.

Descriptive statistics using Kaplan-Meir survival procedures.

Parity Group	N° TrAI	Number censored	Mean	Standard Error	Median Time	Lower 95% CI	Upper 95% CI
Multiparous	26	18	2.436	0.175	3	1.75	5
Nulliparous	40	39	3.613	0.169	4.83	3.17	5
Primiparous	64	26	2.438	0.175	2.05	1.75	2.67
Combined	130	83	3.026	0.118	3.08	2.47	3.73

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	12.3225	2	0.0021*
Wilcoxon	16.3156	2	0.0003*

Table 8. Effect of PG600 dosage level.

Descriptive statistics using Kaplan-Meir survival procedures.

PG600 dosage	N° TrAI	Number censored	Mean	Standard Error	Median Time	Lower 95% CI	Upper 95% CI
0 cc	46	19	2.795	0.204	2.45	2	3.17
1.75 cc	55	51	3.148	0.155	4.5	3.02	5
5 cc	29	13	2.573	0.256	2.25	1.5	3.25
Combined	130	83	3.026	0.118	3.08	2.47	3.73

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	7.2634	2	0.0265*
Wilcoxon	6.0919	2	0.0476*

Table 9. Effect of semen placement site.

Descriptive statistics using Kaplan-Meir survival procedures.

Site (adjusted)	N° TrAI	Number censored	Mean	Standard Error	Median Time	Lower 95% CI	Upper 95% CI
0R	0	48	---	---	---	---	---
1R	0	16	---	---	---	---	---
234R	4	11	3.861	0.391	---	2	5
TrAI	126	8	2.044	0.117	1.83	1.63	2.05
Combined	130	83	3.026	0.118	3.08	2.47	3.73

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	158.9467	3	<.0001*
Wilcoxon	124.6953	3	<.0001*

Table 10. Parameter estimates for the Cox Proportional Hazard reduced model.

Term	Estimate	Std Error	Lower CL	Upper CL
P4 Protocol[N-L]	0.33310436	0.5616534	-0.707126	1.5314056
P4 Protocol [S-N]	-0.9387013	0.4936987	-2.031763	-0.056248
P4 Protocol [XL-S]	1.08845163	0.5174227	0.0686765	2.1016634
Age Group[2-1]	-0.561263	0.2863497	-1.127004	-0.001288
Age Group[3-2]	-0.3956674	0.4445177	-1.300819	0.4449053
Age Group[4-3]	1.04336947	0.4783764	0.1456499	2.031979
Parity[N-M]	-0.4021136	0.4633498	-1.283993	0.5375022
Parity[P-N]	0.90335214	0.2822652	0.361222	1.4699858
SiteAdj[1R-0R]	-0.0582565	433.98166	-850.6467	850.53017
SiteAdj[234R-1R]	13.7619537	376.896	-724.9406	752.46454
SiteAdj[TrAI-234R]	2.03074549	0.5275724	1.1183484	3.239663
PG600[1.75-0]	0.09739015	0.5095552	-0.825909	1.2132304
PG600[5-1.75]	-0.0763584	0.2557198	-0.584799	0.4215234

P4 Protocol: Not synchronized (N), 5-6 d (S), 12-14 d (L) and 24 d (XL).
Age Group: ≤3 years; **2:** >3 and ≤4; **3:** >4 and ≤5; **4:** >5.
Parity: Multiparous (M), Primiparous (P) and Nulliparous (N).
SiteAdj: 0 ring (0R), 1 ring (1R), 2, 3 and 4 rings (234R) and transcervical (TrAI).

Table 11. Risk ratios for age group comparisons.

Parity group comparisons		Risk ratio	Reciprocal
Nulliparous	**Multiparous**	0.6689048	1.4949811
Primiparous	**Multiparous**	1.6507646	0.6057799
Primiparous	**Nulliparous**	2.4678619	0.4052091

Table 12. Risk ratios for site (adj): semen deposition comparisons.

SiteAdj N° of rings (R)		Risk Ratio	Reciprocal
1R	**0R**	0.9434079	1.0599869
234R	**0R**	894211.16	1.1183e-6
234R	**1R**	947852.08	1.055e-6
TrAI	**0R**	6813678.6	1.4676e-7
TrAI	**1R**	7222409.8	1.3846e-7
TrAI	**234R**	7.6197647	0.1312376

Table 13. Risk ratios for PG600 dosage level comparisons.

PG600 dosage levels (cc)		Risk ratio	Reciprocal
1.75	**None**	1.1022903	0.907202
5	**None**	1.0212545	0.9791879
5	**1.75**	0.9264841	1.0793493

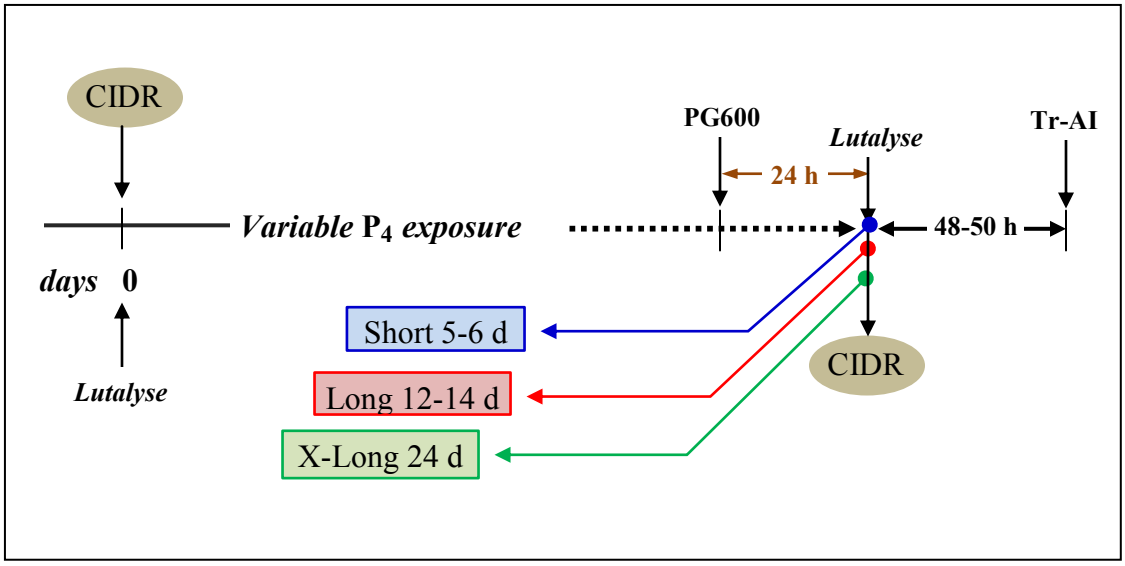

CIDR
Variable P4 exposure
days 0
Lutalyse
PG600
24 h
Lutalyse
Tr-AI
48-50 h
Short 5-6 d
Long 12-14 d
X-Long 24 d
CIDR

Figure 1. Hormonal estrus/ovulation synchronization (E/OS) protocols. Randomly allocated goats to different E/OS protocol groups received an intravaginally placed silicone elastomer CIDR-G® containing 300 mg of P4 and allowed to remain *in situ* for 5-6 d (blue color shaded box), 12-14 d (pink color shaded box), and 24 d (green color shaded box). A 5.0 or 1.75 mL or dose of PG600 5 mL (control group did not receive any PG600) was given 24 h prior to CIDR removal. 2 mL of Lutalyse was given immediately after CIDR removal. 48 to 50 h after P4 removal goats were fixed-time bred using the TrAI procedure.

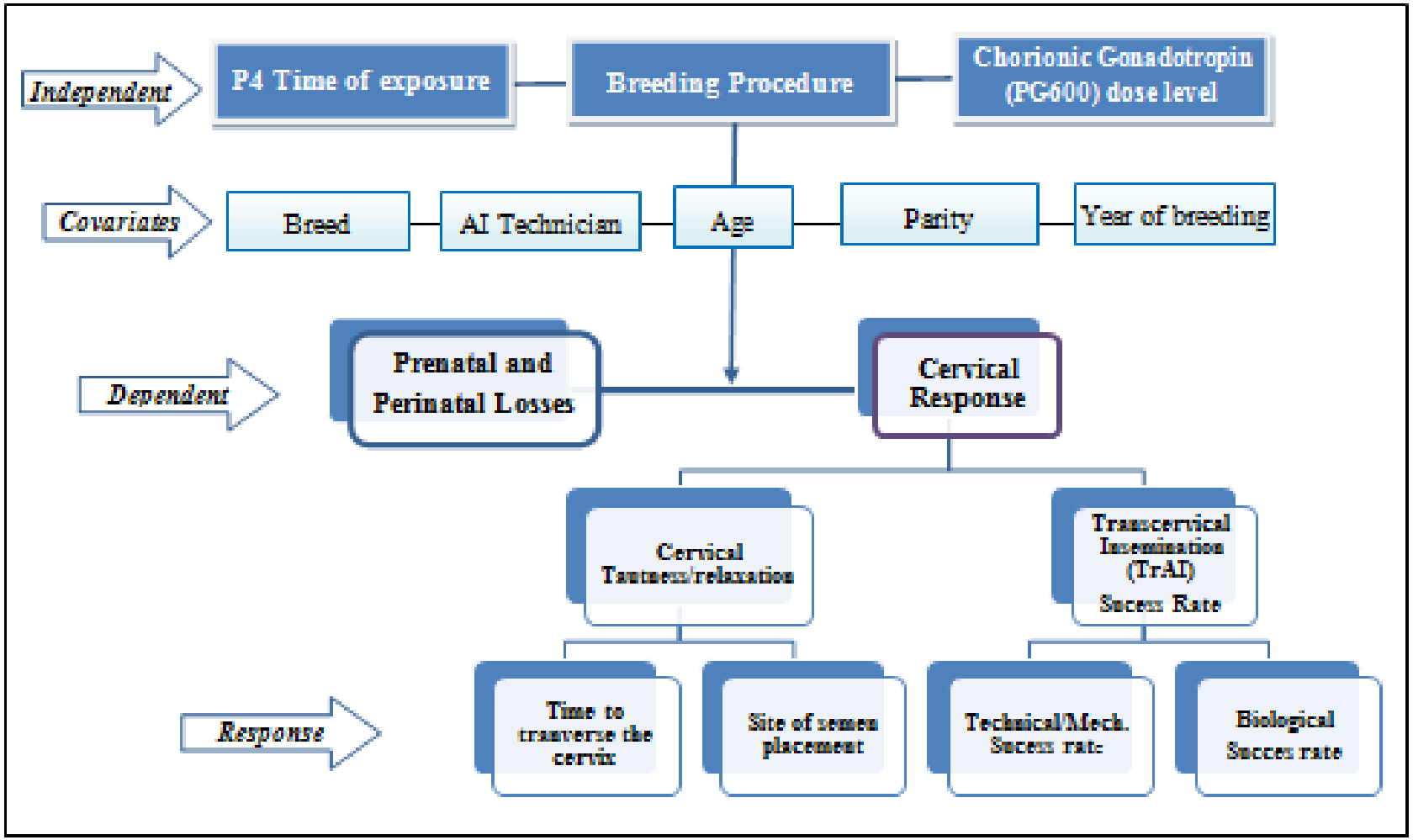

Independent
P4 Time of exposure
Breeding Procedure
Chorionic Gonadotropin (PG600) dose level
Covariates
Breed
AI Technician
Age
Parity
Year of breeding
Dependent
Prenatal and Perinatal Losses
Cervical Response
Cervical Tautness/relaxation
Transcervical Insemination (TrAI) Sucess Rate
Response
Time to transverse the cervix
Site of semen placement
Technical/Mech. Sucess rate
Biological Succes rate

Figure 2. Relationship of study variables. Variables considered were: independent, covariates, dependent and response variables.

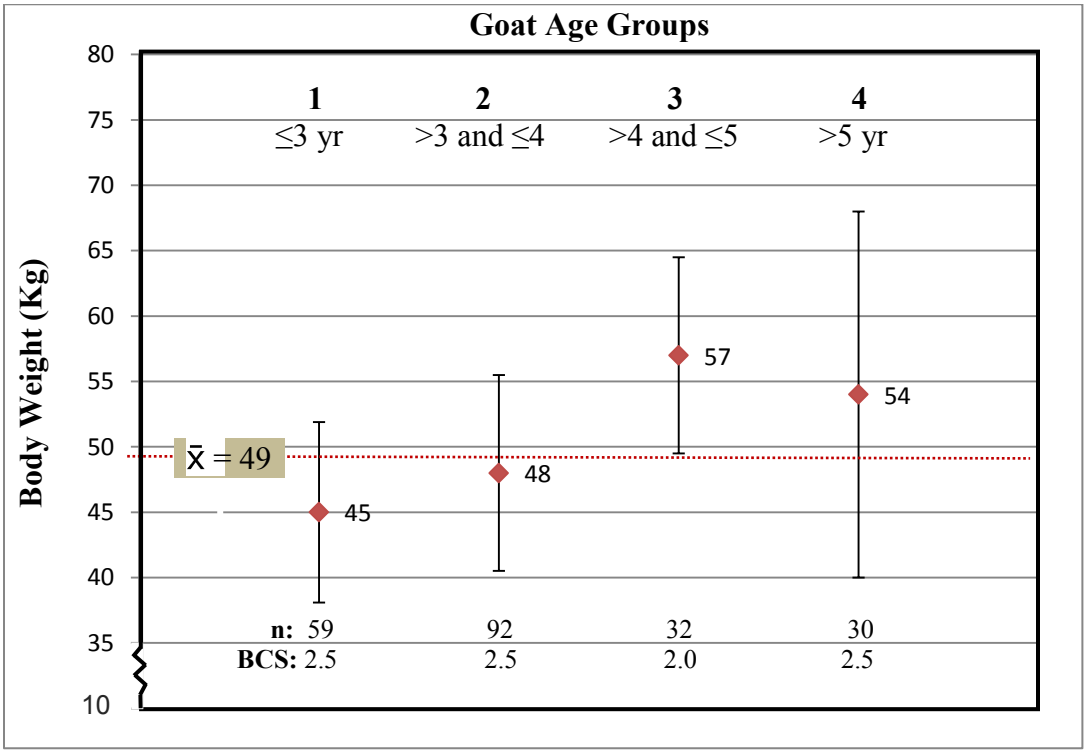

Goat Age Groups
Body Weight (Kg)
80
75
70
65
60
55
50
45
40
35
10
1
≤3 yr
2
>3 and ≤4
3
>4 and ≤5
4
>5 yr
x̄ = 49
45
48
57
54
n: 59 92 32 30
BCS: 2.5 2.5 2.0 2.5

Figure 3. Mean body weight ±SD by age group, sample size and body condition score (BCS) at first breeding. Goats of different breeds (Alpine, Angora, Boer, Spanish, and Tennessee Stiff legs), parity categories, and ages were used in this study. The overall average weight at first breeding was 49.1 ±9.58 kg. The most common BCS on a scale of 1 to 5 was 2.5. . Goat ages ranged from1.5 to 10 y of age. The overall average age and standard deviation at breeding time was calculated to be 3.7 ±1.4 y .

	XL	S	N	L	Total
NoTrAI	13	30	19	17	79
TrAI	12	32	40	50	134
%TrAI▶	48%	52%	68%	75%	63%

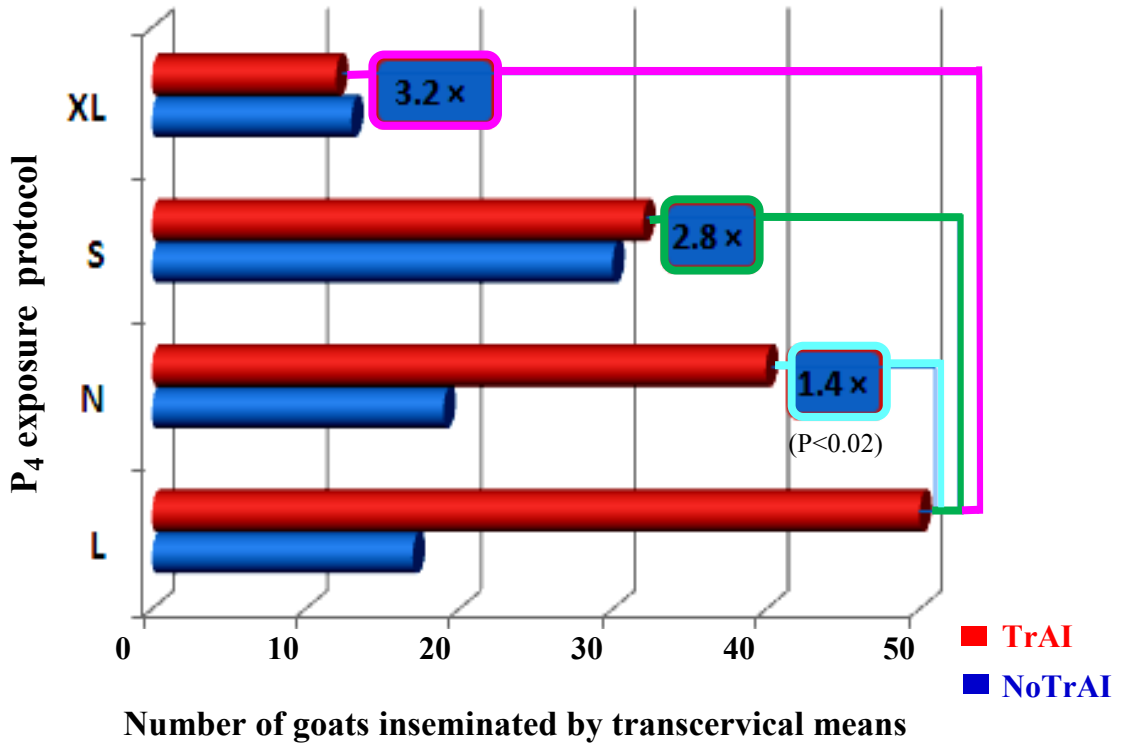


Figure 4. Transcervical artificial insemination mechanical/technical success rate (TrAI $SR_{m/t}$) as a function of P4 exposure. Short P4 (S:5-6 d), long P4 (L:12-14 d), extra l-long P4 (XL: 24 d), and no P4 (N; control). Label displayed reflects number of times (×) greater likelihood of a successful TrAI (blue bars) compared to no-TrAI (red colored bars). For example, 12 d P4 exposure protocol with a TrAI $SR_{m/t}$ of 75% was greater ($P<0.02$) than goats inseminated during a natural estrus with no hormone synchronization protocol (TrAI $SR_{m/t}$ of 68%). Other main effect comparisons showed that there were differences between the non-treated control goats compared with the P4 exposure protocols.

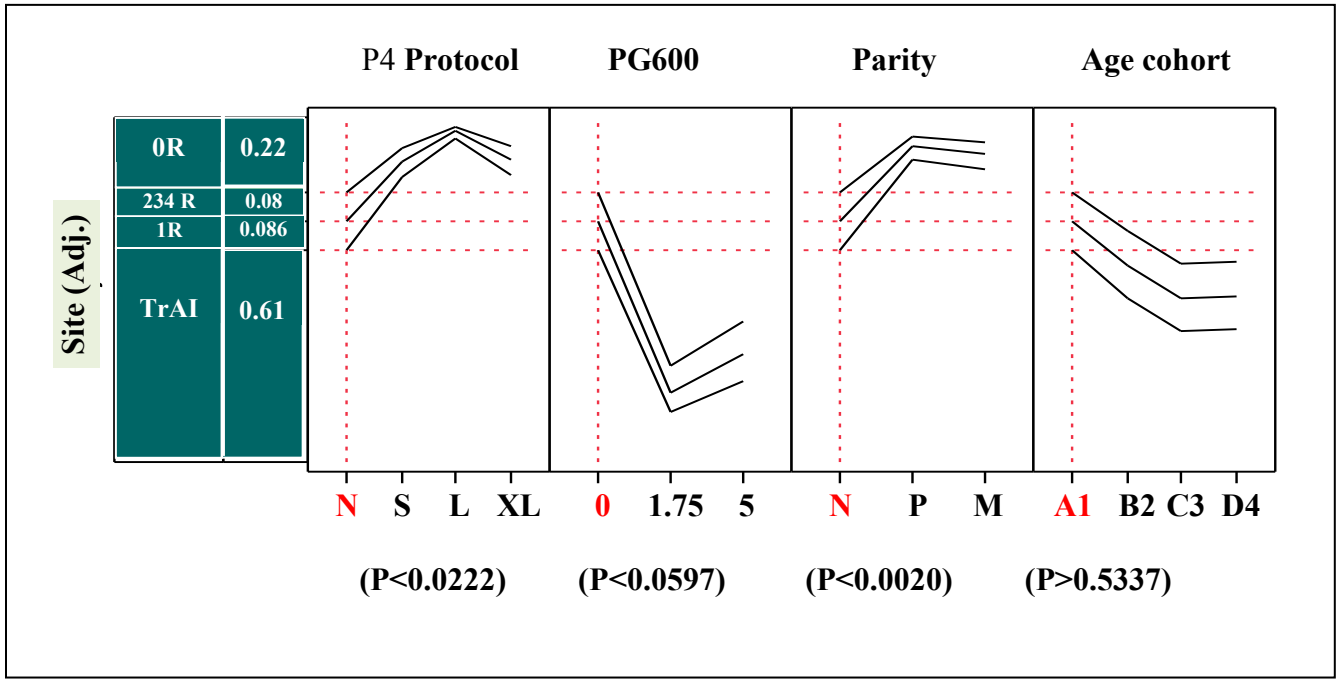

P4 Protocol
PG600
Parity
Age cohort
Site (Adj.)
0R
0.22
234 R
0.08
1R
0.086
TrAI
0.61
N S L XL
0 1.75 5
N P M
A1 B2 C3 D4
(P<0.0222)
(P<0.0597)
(P<0.0020)
(P>0.5337)

Figure 5. Predicted profiles for the influence of independent variables on semen site deposition with associated probabilities (P) based on fitting data to the ordinal logistic model [2]. *P4 exposure protocol:* N=None, S= 5-6 d, L= 10, 12, 14 d, and XL= 24 d; *PG600* : 0.0, 1.75, 5.0 mL; *Parity:* N= nulliparous, P= primiparous, and M= multiparous; *Age cohort:* A1≤3 y, B2>3 and ≤ 4 y, B3>4 and ≤5, and B4>5. Left green boxes show coefficient of success at each cervical site (R= cervix ring): ∅R, 1R, 2, 3, 4R and TrAI (complete traverse of cervix). For example a nulliparous goat, 3 or less years old goat which was not synchronized the model used [2] anticipates a 61% TrAI success rate.

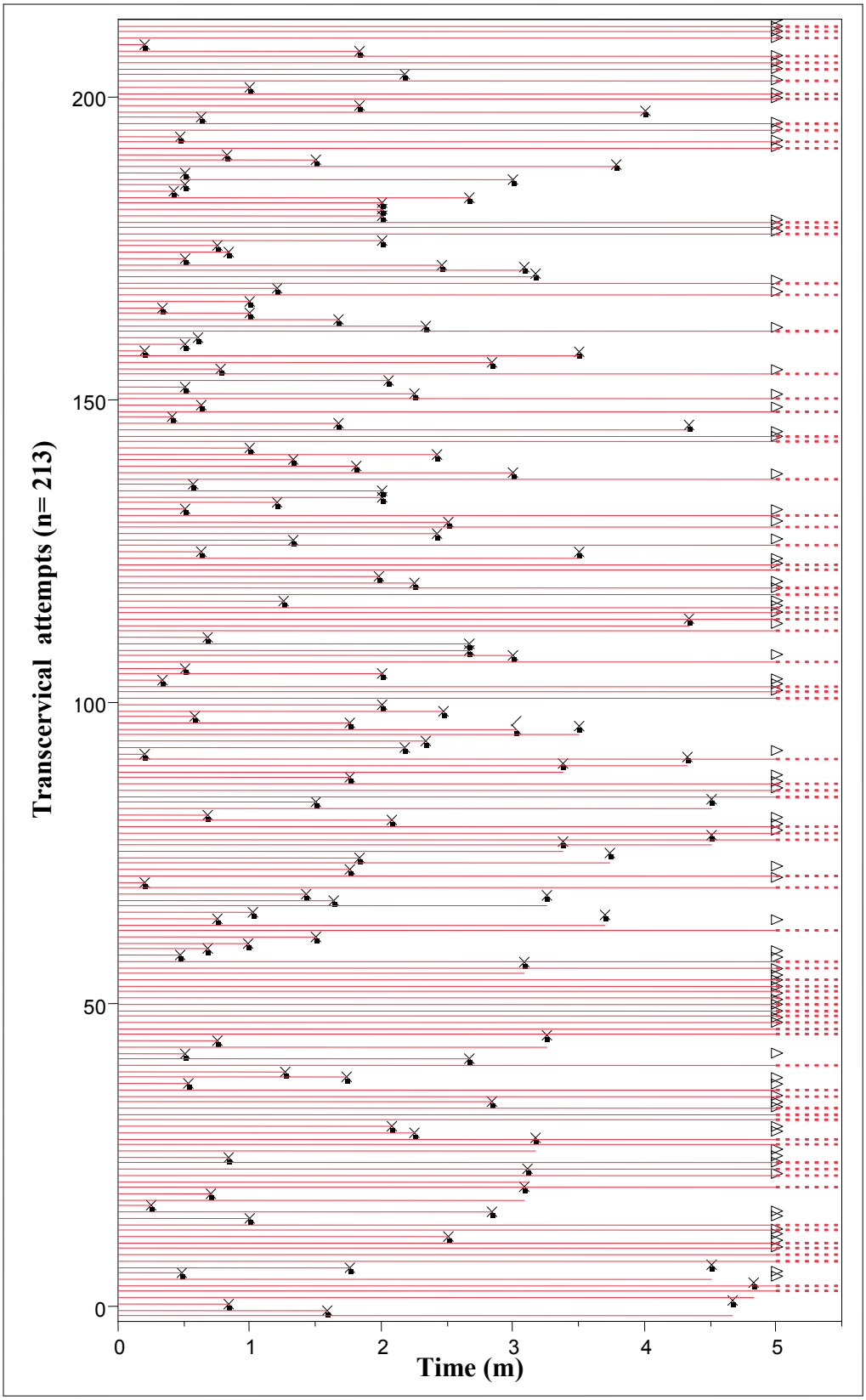
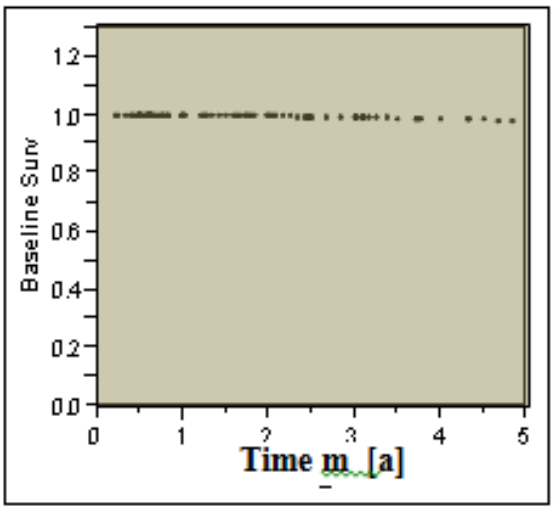

Transcervical attempts (n= 213)
200
150
100
50
0
0
1
2
3
4
5
Time (m)

Figure 6. Transcervical attempt event plot. The entry of goats to the experiment was staggered. The clinical survival end-point was determined to be a successful transcervical artificial insemination (TrAI) measured in minutes (m). The plot displays all 213 time measurements with 83(39%) administratively right censored at 5 m. The y-axis shows individual breeding attempts. A solid line not followed by a dashed line marked by an "x" at the end of the line indicates a successful TrAI event. A dashed line connecting to the right of a solid line with a left triangle indicates a right-censored event.


Baseline Surv
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
Time m_[a]

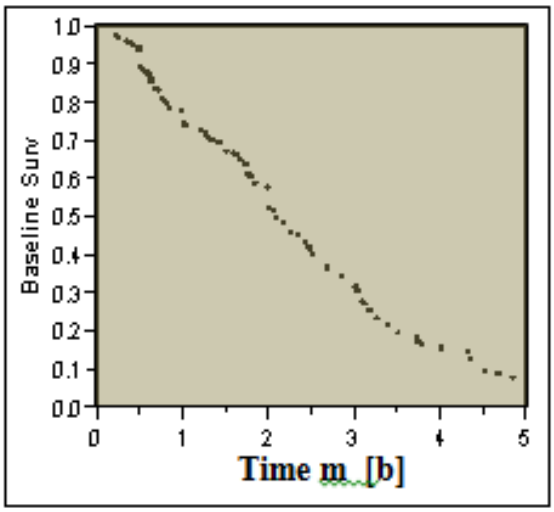

Baseline Surv
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
Time m_[b]

Figure 7. Baseline survival at mean values for all independent variables. Panel [a] corresponds to a reduced model with all cervical rings included. Panel [b] represents a reduced model without sites RØ and R1. As time increases the probability of a TrAI also decreases because more difficult does to traverse the cervix take longer time to be successful.

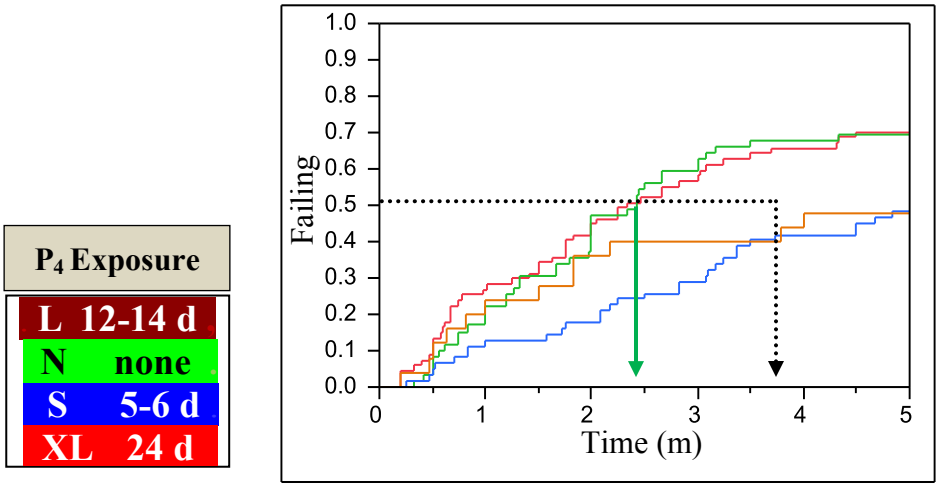

P4 Exposure
L 12-14 d
N none
S 5-6 d
XL 24 d
Failing
Time (m)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
1
2
3
4
5

Figure 8. Failure plot of TrAI response to P4 exposure treatments. The length of time goats were exposed to P4 had an influence on the time it took to penetrate a goat's cervix. As a group all goats had a median TrAI time of 3.08 m (dotted black downward arrow). The median value where 50% of goats that were not treated (N) or that were estrus/ovulation synchronized using P4 for 12 to 14 d were TrAI'ed in about 2.4 m (solid green downward arrow).

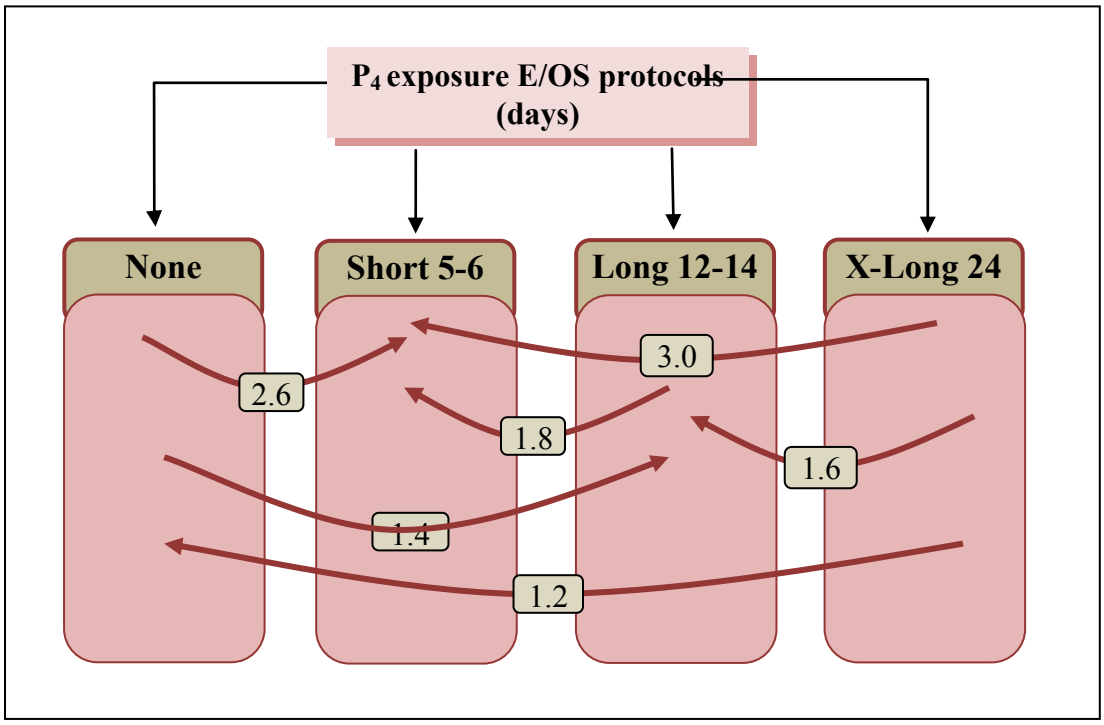

P4 exposure E/OS protocols (days)
None
Short 5-6
Long 12-14
X-Long 24
2.6
3.0
1.8
1.6
1.4
1.2

Figure 9. Risk ratios for P4 exposure synchronization protocol contrasts. Six possible group mean comparisons are provided with the number inside the box representing the risk ratio value (RR). The arrowhead direction establishes the nature of the RR comparison. Left to right (⟶) = Left P4 treatment had more TrAI and *vice-versa*.

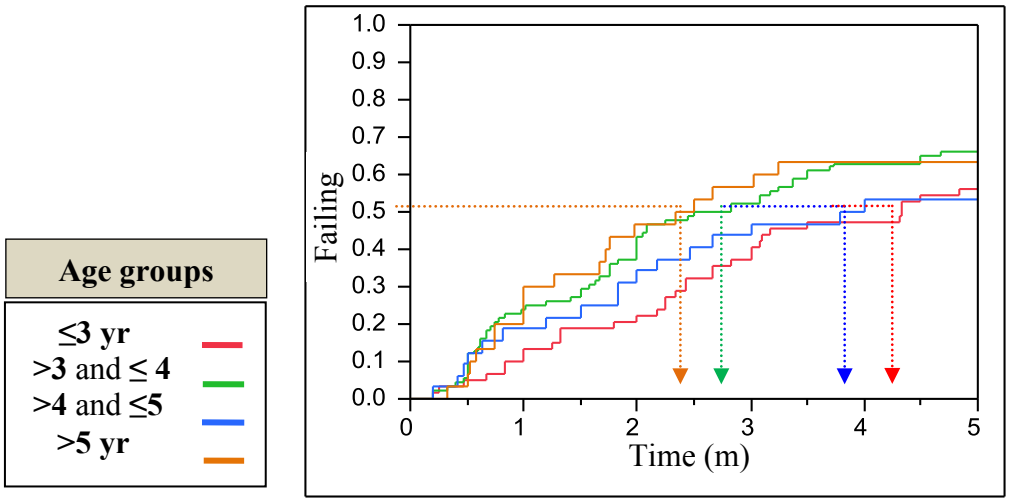

Age groups
≤3 yr
>3 and ≤ 4
>4 and ≤5
>5 yr
Failing
Time (m)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
1
2
3
4
5

Figure 10. Failure plot of TrAI response to goat age groups. Each color coded downward arrow depicts the median value where 50% of goats that were categorized in a given age groups were successfully TrAI'ed. Older goats (5 y) were more likely to get inseminated earlier at about 2.5 m after the procedure was initiated rather than 3.9 m and 2.8 m for younger goats at >4 and ≤5 y and >3 and ≤4 y, respectively. Fifty percent of the young goats (≤3 y) were TrAI'ed about 1.8 m later (a change of 42%) than it took to TrAI goats 5 y or older.

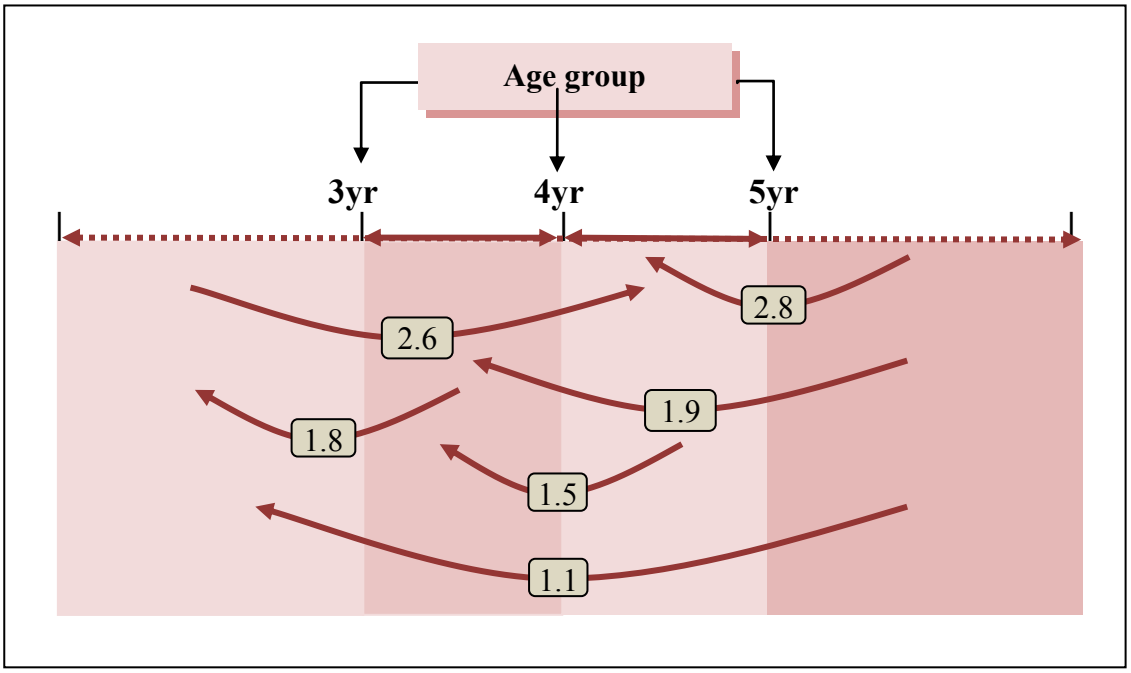

Age group
3yr
4yr
5yr
2.6
2.8
1.8
1.9
1.5
1.1

Figure 11. Risk ratios for age group contrasts. Six possible age group mean comparisons are provided with the number inside the box representing the risk ratio value (RR). The arrowhead direction establishes the nature of the RR comparison. Right to left (⟵) = Right age group treatment had more TrAI and *vice-versa*.

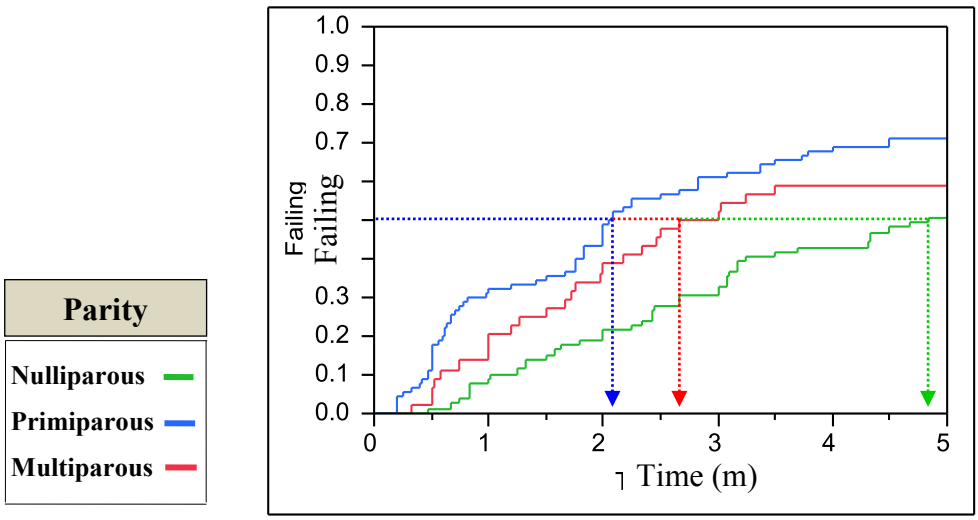

Parity
Nulliparous
Primiparous
Multiparous
Failing
Failing
1.0
0.9
0.8
0.7
0.3
0.2
0.1
0.0
0
1
2
3
4
5
Time (m)

Figure 12. Failure plot of TrAI response to goat parity category. The grouping to which goats were categorized in terms of parity influenced the time it took to completely penetrate a goat's cervix. As a group all goats had a median TrAI time of 3.03 m. Half of the primiparous goats were likely to have had transcervical insemination almost 3 m sooner and almost 2 m before than 50% of nulliparous goats. Primiparous and multiparous goats were not different in respect to the median number of goats inseminated at a given time.

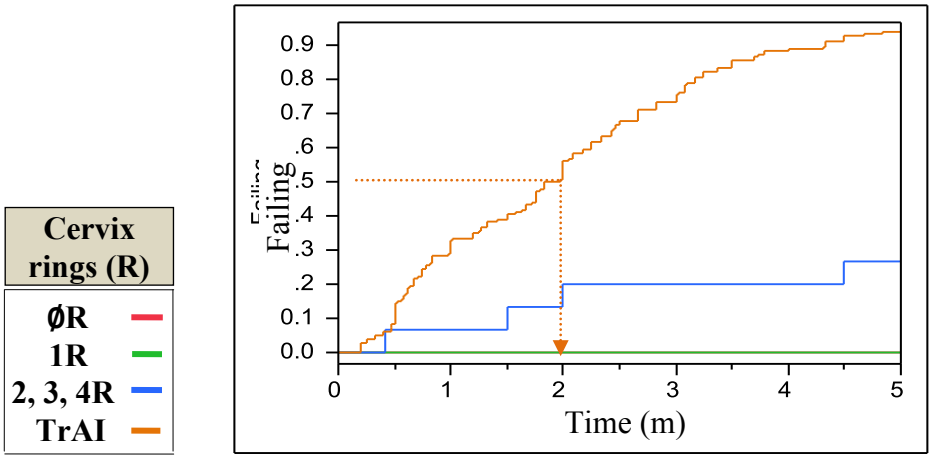
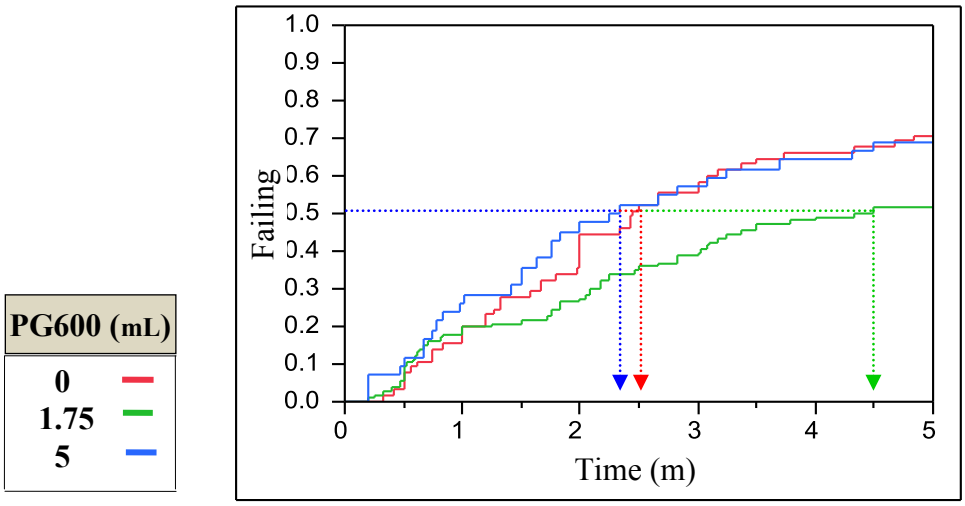
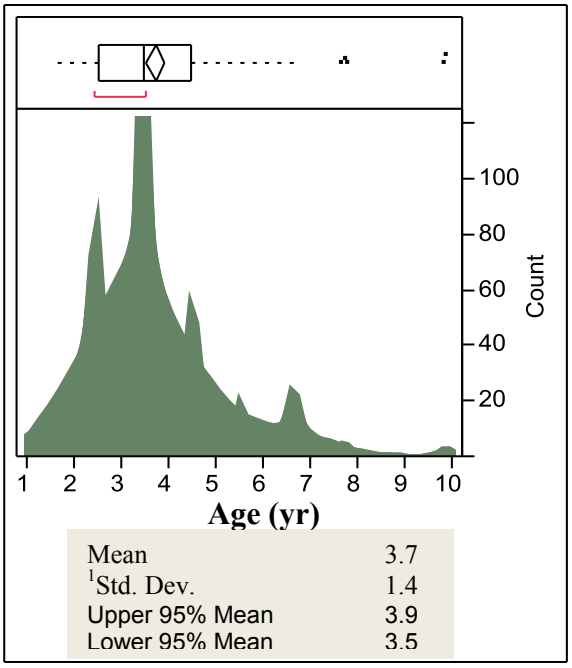

Cervix rings (R)
ØR
1R
2, 3, 4R
TrAI
Failing
0.9
0.8
0.7
.6
.5
.4
.3
.2
0.1
0.0
0
1
2
3
4
5
Time (m)

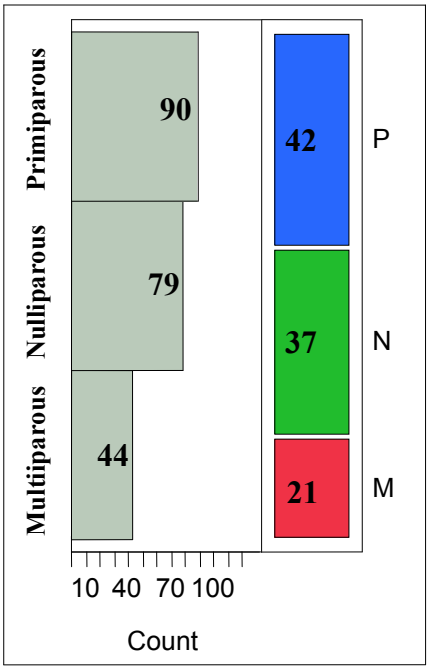
Figure 13. Failure plot of TrAI response to semen cervical placement. The site where semen was placed in the goat's cervix influenced the time to completely penetrate a goat's cervix. The step function corresponding to ØR and 1R is biased. For this reason both cumulative distributions do not appear in the failure plot as both had all their observation at 0 RR value. Fifty percent of the goats that had a successful insemination were finished in 1.8 m.


PG600 (mL)
0
1.75
5
Failing
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
Time (m)

F**igure 14.** Failure plot of TrAI response to PG600 dose level. Goats in the control group and goats receiving 5.0 cc of PG600 appear to be more efficient than the 1.75 cc PG600 dose protocol. The latter almost doubled the time to TrAI. However, PG600 was not influential.


100
80
60
40
20
Count
1 2 3 4 5 6 7 8 9 10
Age (yr)
Mean 3.7
[1]Std. Dev. 1.4
Upper 95% Mean 3.9
Lower 95% Mean 3.5

[a]


Primiparous
Nulliparous
Multiparous
90
79
44
42
37
21
P
N
M
10 40 70 100
Count

[b]

Figure 15. Goat age shadowgram distribution [a] and histogram of parity groups [b]. In panel [a] there are 5 peaks at corresponding to ages 2.5, 3.7, 4.5, 5.5, and 6.8 years of age. In panel [b] 90 goats (42%) were classified as primiparous (P), 79 were nulliparous (37%; N). and 44 were multiparous (21%; M).

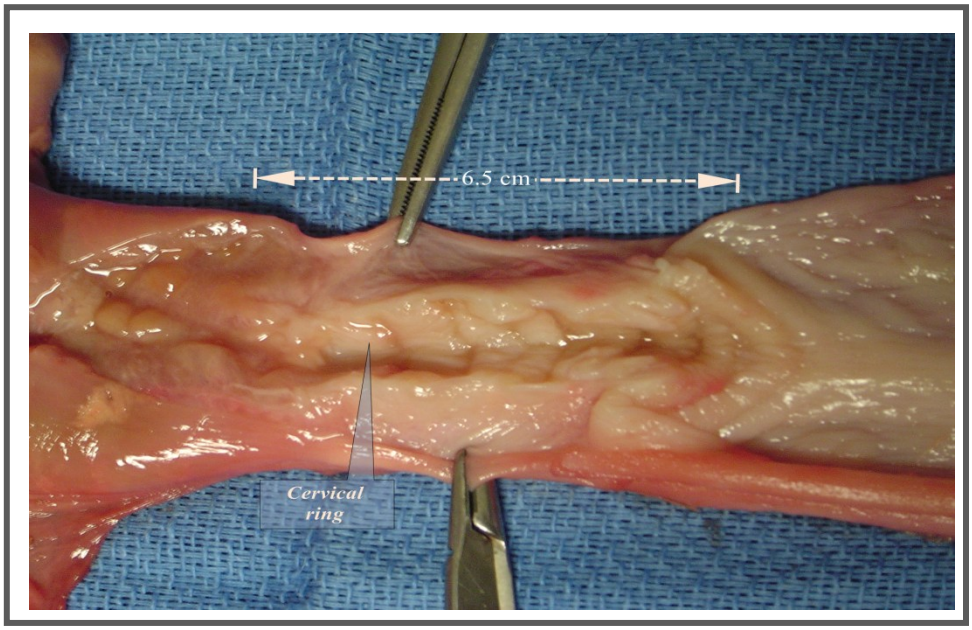

6.5 cm
Cervical ring

Image 1. Goat cervix. A total of 5-6 cervical tissue fold (rings) are visible in a cervix that measures 6.5 cm. External os cervix is rightmost nest to the caudal vestibule of the vagina. Leftmost section shows part of the uterine body with caruncular tissue

VITA

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Candidate for the Degree of

Doctor of Philosophy

Dissertation: **REPRODUCTIVE PERFORMANCE, EARLY PROGENY WASTAGE, AND CERVIX RESPONSE USING FIXED-TIME INTRAUTERINE OR TRANSCERVICAL INSEMINATION OR NATURAL SERVICE FOLLOWING SYNCHRONIZATION OF ESTRUS AND OVULATION IN GOATS**

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Pages in Study: 406 Candidate for the Degree of Doctor of Philosophy

Major Field: Veterinary Biomedical Science.

Scope and Method of Study:
Assisted reproductive technologies provide necessary tools for improving farm revenue. Hormonal estrus and ovulation synchronization, while decreasing costs of production and allowing for economies of scale advantage, reduce reproductive efficiency. A randomized experimental prospective field and clinical trial using ultrasound imaging was conducted to determine the effect of estrus/ovulation synchronization protocol on goat reproductive performance, prenatal and perinatal losses, and cervix response of dairy, meat and fiber production phenotypes using fixed-time insemination by different breeding procedures.

Findings and Conclusions:
Compared to natural service most reproductive efficiency traits used to describe goat reproductive performance were negatively influenced by the assisted reproductive technologies implemented. When hormonal estrus/ovulation synchronization protocols are used in conjunction with fixed-time breeding initial acceptable conception rates are reduced by time of parturition, hence kidding rates are lower across breeds, ages, and parity categories. The decrease in reproductive performance is mainly due to short P4 exposure combined with fixed-time breeding rather than concurrent use of eCG and hCG, although the use of the chorionic gonadotropins resulted in high early progeny wastage, particularly embryonic mortality. Goats displayed a pattern of early rather than late progeny loss. Prenatal losses were influenced by: breed, age and time of exposure to P4. Increased prenatal losses were influenced by breeding procedures particularly excessive manipulation during trans-cervical artificial insemination. Short P4 exposure increased breeding time investment, made less likely to traverse the cervix, and thus influenced the site of semen deposition. The use of real-time ultrasound imaging for pregnancy diagnosis at 45 days post-breeding resulted in high sensitivity, accuracy, and precision. However, the technology was not reliable to establish the number of embryos in non-tractable goat production phenotypes and/or parity categories under field conditions.

ADVISER'S APPROVAL: Dr. Jerry Malayer